REVIEW

Natural Products for Cancer-Targeted Therapy: Citrus Flavonoids as Potent Chemopreventive Agents

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Abstract

Targeted therapy has been a very promising strategy of drug development research. Many molecular mechanims of diseases have been known to be regulated by abundance of proteins, such as receptors and hormones. Chemoprevention for treatment and prevention of diseases are continuously developed. Pre-clinical and clinical studies in chemoprevention field yielded many valuable data in preventing the onset of disease and suppressing the progress of their growth, making chemoprevention a challenging and a very rational strategy in future researches. Natural products being rich of flavonoids are those fruits belong to the genus citrus. Ethanolic extract of Citrus reticulata and Citrus aurantiifolia peels showed anticarcinogenic, antiproliferative, co-chemotherapeutic and estrogenic effects. Several examples of citrus flavonoids that are potential as chemotherapeutic agents are tangeretin, nobiletin, hesperetin, hesperidin, naringenin, and naringin. Those flavonoids have been shown to possess inhibition activity on certain cancer cells' growth through various mechanisms. Moreover, citrus flavonoids also perform promising effect in combination with several chemotherapeutic agents against the growth of cancer cells. Some mechanisms involved in those activities are through cell cycle modulation, antiangiogenic effect, and apoptosis induction.Previous studies showed that tangeretin suppressed the growth of T47D breast cancer cells by inhibiting ERK phosphorylation. While in combination with tamoxifen, doxorubicin, and 5-FU, respectively, it was proven to be synergist on several cancer cells. Hesperidin and naringenin increased cytotoxicitity of doxorubicin on MCF-7 cells and HeLa cells. Besides, citrus flavonoids also performed estrogenic effect in vivo. One example is hesperidin having the ability to decrease the concentration of serum and hepatic lipid and reduce osteoporosis of ovariectomized rats. Those studies showed the great potential of citrus fruits as natural product to be developed as not only the source of co-chemotherapeutic agents, but also phyto-estrogens. Therefore, further study needs to be conducted to explore the potential of citrus fruits in overcoming cancer

Keywords: Natural products - targeted therapy - cancer - citrus flavonoids - co-chemotherapy - chemoprevention

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Introduction

Disease is the term commonly used to define a condition when impair of normal function occurs, both infectious and not. As a consequence of the dysfunction, in some cases, signs that are referred to medical symptoms appeared. Not only affecting and altering one's life, these symptoms are also reducing quality of life, and moreover, might cause death. Globalization resulted certain impacts on people's lifestyle, thus their health (Epperly et al., 2011). Exposure to UV rays and smoke cause various health disorders, such as hyperpigmentation, aging process, and increasing of skin and lung cancer risk (Elmets et al., 2010). Therefore, chemoprevention as an effort for treatment and prevention of diseases are continuously developed. As an examples are the use of antioxidants and anti-aging substances (Epperly et al., 2011).

Chemoprevention is defined as the use of substances of natural origin, biological agents, synthetic, or chemical compounds to prevent or suppress the disease progression, reverse to normal physiological functions and perform early detection of pathological conditions (Hirsch et al., 2002; Tsao et al., 2004). Chemopreventive agents, therefore, are expected to overcome several problems, such as skin cancer (melanoma), osteoporosis, and degenerative diseases (Lin & Tsai, 1999; Uliasz & Spencer, 2004). Pre-clinical and clinical studies in this field has yielded many valuable data in preventing the onset of disease and suppressing the progress of their growth, making chemoprevention a challenging and a very rational strategy in future researches (Adhami et al., 2009; Bishahee et al., 2011).

Treatment of cancer, a disease with a complex molecular pathophysiology that varies according to each type, has been facing huge number of problems. Several ways in the treatment of breast cancer have been developed, that are surgery, chemotherapy, hormonal therapy, and radiation. The use of systemic chemotherapy does not show satisfying result, and even causes side

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effects (Wattanapitayakul et al., 2005). The usage of doxorubicin, a chemotherapeutic agent commonly used in breast cancer treatment, showed low effectivity, rendering to its resistance and toxicity on normal tissues (Fimognari et al., 2006). An approach in overcoming such problem is the development of agents used in combination with chemotherapeutic agents resulting in better result compared to chemotherapeutic agent alone (Fimognari et al., 2006). Therefore, study focusing on cancer chemoprevention has to be continuously conducted.

Another disease showing continuous increasing incidence are hypercholesterolemia and osteoporosis, particularly suffered by women entering their menopause phase. Those are a couple of common problems faced by women commonly linked to cancer development, because they are related to estrogen and estrogen receptor modulation (Yager & Davidson, 2006). Such hormones are being responsible in women's physiological system, that are maintaining menstrual cycle, reproductive tissues, bone density, and also cholesterol modulation (Jordan, 2004). Estrogen deficiency may lead to disruption in reproductive tissues, increasing risk of cardiovascular dysfunction, hot flashes, and insomnia (Lewis-Wambi & Jordan, 2005). Since ER activation leads to the activation of replication machineries, people's effort to overcome menopause and osteoporosis by using hormone replacement therapy brings along a higher risk of cancer, particularly breast and endometrial cancer (Yager & Davidson, 2006). Hormone replacement therapy is the use of exogenous estrogen in order to maintain certain normal functions in the body. The use of phytoestrogens as a replacement of estradiol being used in HRT to decrease cancer risk is then developed, thus categorized as chemopreventive agents (Shao & Brown, 2003). Not only phytoestrogens, compounds possessing antioxidant activity are also potential as chemopreventive agents (Seely et al., 2005).

As mentioned above, chemoprevention is one promising approach in overcoming cancer problems. Another main problems being faced in the application of chemotherapeutic agent are its toxicity towards normal tissues, suppression of the immune system, and occurrence of resistance (Mechetner et al., 1998; Wattanapitayakul et al., 2005). One promising approach to solve this problem is the application of cochemotherapeutic agent in cancer therapy (Brenner, 2002). Co-chemotherapy may increase chemotherapeutic agents' efficacy, allowing the use of lower dosage of chemotherapeutic agent, resulting in the decrease of toxicity on normal tissues (Bastl et al., 2007). Increasing efficacy may be obtained by combining agents with established chemotherapeutic agents having additive or synergistic effect, while decreasing the toxicity may be achieved by the use of agents possessing immunomodulatory effect (Olson et al., 2007).

Citrus species is one example of natural product containing phytochemicals that is promising to be developed in cancer therapy. Numbers of studies have been conducted, providing information related to citrus' potential as both chemopreventive and cochemotheurapetic agent. Here, we would like to explore citrus as a model of natural product to be developed for both preventing and overcoming cancer. Discussion would be focusing on two members of *Citrus* family, that are *Citrus aurantifolia* and *Citrus reticulata*.

Chemopreventive Properties of Citrus Flavonoids

Cancer Chemoprevention Research Center (CCRC), Faculty of Pharmacy Universitas Gadjah Mada has explored several agents possessing biological activity related to cell growth and differentiation, either in the form of synthetic compounds or plant extracts. CCRC has explored at least two member of citrus family, e.g Citrus aurantiifolia and *Citrus reticulata* (Table I) and biological activities of their flavonoids, that are hesperidin, naringenin, naringin, nobiletin, and tangeretin (Table II). In combination with another studies conducted, those could be a very valuable information to be analyzed further as the source of information for future studies.

Antioxidant Activity

Antioxidants are agents possessing ability to interact with and stabilize free radicals, thus resulting in protection of cell damage. Since free radical DNA damage may cause cancer, antioxidants are then proposed to be able to slow and prevent further damage in the development of cancer. Though DNA oxidation and carcinogenesis have not been directly proven, studies gave indirect strong support of certain product of DNA oxidation acting as biomarker for assessing antioxidant status and cancer risk (Thompson, 2004).

Citrus peels has been known to be a potential natural antioxidants because of their phenolic and flavonoid compounds (Bocco et al., 1998; Li et al., 2006). *C. aurantiifolia* extract containing hesperidin as the most dominant flavonoid showed radical scavenging activity (Tumbas et al., 2010). Hesperidin was also proven to be moderately active as antioxidant detected by using electron spin resonance spectrophotometer (Al-Ashaal & El-Sheltawy, 2011). Hesperidin, nobiletin, and tangeretin showed antioxidant activity in various antioxidant assays *in vitro* (Yi et al., 2008).

Suppression of Carcinogenesis

Carcinogenesis is the process of initiation and promotion of cancer. The processes involved are complex, affected by genetics factors, environmental exposure, age, and others. For cancer cells to initiate and thrive, they have to be able to stimulate their growth, gather enough factors required, and avoid elimination mechanisms. Chemopreventive agents are compounds that are able to prevent, slow down, or stop carcinogenesis (Tamimi et al., 2005).

In vivo study showed that besides suppressing c-Myc expression, C. reticulata peels ethanolic extract with the dosage of 500 mg/kgBW might reduce the number of cells expressing N-Ras in DMBA-induced rats' hepatic carcinogenesis (Putri et al., 2008; Nugroho et al., 2008) and its suppression of c-Myc expression as well. Nobiletin, flavonoid obtained from *C. reticulata* peels, blocked

carcinogenesis on DMBA-induced mice's skin (Murakami et al., 2000).

Further study was then conducted via in silico study on nobiletin and tangeretin, two flavonoids contained in *C. reticulata* peels (Johann et al., 2007). Those two citrus flavonoids do not bind c-Src (N-Ras' upstream) stronger that ATP and imatinib do, respectively, while imatinib-c-Src has more stable binding compared to that is on ATP-c-Src (Nugroho et al., 2008; Putri et al., 2008). Molecular docking of nobiletin and tangeretin on CYP1A2 (isoenzyme of chytochrome P-450 that is able to activate procarcinogenic substance, benzo[A]pyrene) showed that tangeretin binds CYP1A2 stronger than α-naphtoflavon, while nobiletin does not (Nugroho et al., 2010). Those *in silico* studies should be further conducted, in order to predict the mechanism of action of certain molecules, and also to be used as the basic information in the discovery of new active molecules for future researches.

While *C. aurantiifolia*, another member of citrus family, showed inhibition of mammary carcinonegenis of DMBA on female sprague sawley rats by apoptosis induction and inhibition of cell proliferation (Hastuti et al., 2008; Pratiwi et al., 2008). *Citrus aurantifolia* peels contain some flavonoids such as naringin, hesperidin, naringenin, hesperitin, rutin, nobiletin, dan tangeretin (Choi et al., 2007). Twenty mg/kg BW hesperetin per oral daily administration for 15 weeks inhibit carcinogenesis on rat's colon during and after initiation of 20 mg/kg BW 1,2 dimethylhydrazin (Aranganathan and Nalini, 2009).

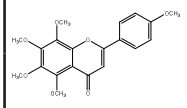
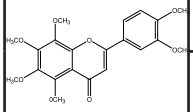
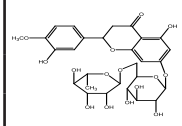
Effect on Cell Cycle Regulation

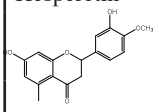
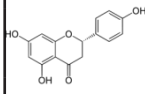
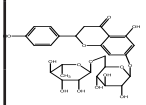
Cancer is described as a disease produced by damage to cell cycle regulation. The presence of damage or mutations in the cells should stimulate cell death, but in cancer cells this phenomenon does not occur and the cell stays alive through the cell cycle with the accumulation of mutations in the cell. Characteristics of cancer cells one of which is the ability to grow without stimulation of growth due to changes in intracellular signaling pathways that cause cells to be able to enter the cell cycle with no need of positive or negative external stimuli (Foster, 2008). Stimulation of growth began with the release of growth factors from the cell. Furthermore, these growth factors will bind to receptors on the cell membrane. Signals transmitted through the membrane into the cytoplasm and transcription factors resulted in the release of subsequent protein in the cell nucleus. It encourages cells to enter the cell cycle. Cells that will replicate undergo G1 phase (first gap phase) to S phase for DNA synthesis. S phase is followed by the G2 phase, where the cell prepares for duplicating equipments for M (mitosis) phase (Foster, 2008).

Table 1. Chemopreventive effect of Citrus Ethanolic Extract

Extract	Chemopreventive Effect	Biological Effect	Reference
Citrus reticulata peels (ethanolic)	Antioxidant	Scavenge DPPH radicals and hydroxyl radicals, stabilizing sunflower oil during accelerated storage	Tumbas et al., 2011
	Supression of carcinogenesis	Suppress N-Ras and c-Myc expression in rats' hepatic carcinogenesis Antiproliferative on rats' mammary carcinogenesis with increase of p53 expression	Nugroho et al., 2008 Putri et al., 2008] Pratomo et al., 2008
	Apoptosis	Antiproliferative on rats' hepatic carcinogenesis by inducing apoptosis with increase of p53 expression	Pratomo et al., 2008
	Angiogenesis and metastasis	Inhibiting new blood vessels formation and reduce number of macrophage cells on bFGF-induced chicken CAM's embryo Proliferative and increase VEGF expression on WiDr cells	Chrisnanto et al., 2008 Ardiani et al., 2008 Puspita et al., 2008
	Estrogenic	Proliferative on MCF-7 cells Induce rats' mammay gland development and increase uterus' volume by increasing c-Myc expression; increase bone density and improve blood cholesterol profile Proliferative on WiDr cells n low concentrations	Yunas, et al., 2007 Supriyati et al., 2008 Adelina et al., 2008a; 2008b Ardiani et al., 2008
Citrus aurantifolia peels (ethanolic)	Supression of carcinogenesis	Inhibited MCF-7 cells growth Inhibited proliferation of female sprague dawley rat mammary induced by DMBA by downregulation of c-myc	Adina et al., 2008 Pratiwi et al., 2011
	Cell cycle regulation	Modulate MCF-7 cell cycle	CCRC, unpublished data
	Apoptosis	Induced Apoptosis on female sprague dawley rat mammary induced by DMBA by upregulation of p53 Increased apoptosis induction of doxorubicin by upregulation of p53 and down regulation of Bcl-2	Hastuti et al., 2008 CCRC, unpublished data
	Co-chemotherapeutic	Increased cytotoxicity of doxorubicin on MCF-7 cells	Adina et al., 2008 CCRC, unpublished data

Table 2. Chemopreventive effect of Citrus Flavonoid on Molecular Targets

Phytochemical and Chmical Structure	Chemopreventive Effect	Molecular Targets	Biological Effect	Ref.
Tangeretin 	Antioxidant		Scavenge DPPH radicals, hydroxyl radicals, superoxide anion radicals, hydrogen peroxides, and possess reducing power	Yi et al., 2008
	Supression of carcinogenesis	c-Src CYP1A2	Inhibit the activity (in silico study)	Nugroho et al., 2008; 2010 Putri et al., 2008
	Cell cycle regulation	p53, p21, p37 -	Upregulation on COLO 205 cells G1 arrest on MCF-7 cells	Pan et al., 2002 Morley et al., 2007
	Apoptosis	p53 -	Trigger apoptosis in COLO 205 cells Induce apoptosis on HL-60 cells	Pan et al., 2002 Hirano et al., 1995
	Angiogenesis and metastasis	ERK-2 HIF1-α	Inhibit the activity (in silico study)	Puspita et al., 2008
	Estrogenic	-	-	-
	Co-chemotherapeutic	-	Increased cytotoxicity of doxorubicin on MCF-7 and T47D cells	Meiyanto et al., 2011
Nobiletin 	Antioxidant	-	Scavenge DPPH radicals, hydroxyl radicals, superoxide anion radicals, hydrogen peroxides, and possess reducing power	Yi et al., 2008
	Supression of carcinogenesis	c-Src CYP1A2	Inhibit the activity (in silico study)	Nugroho et al., 2008; 2010 Putri et al., 2008
	Cell cycle regulation	- -	Modulate cell cycle on TMK-1, MKN-45, MKN-74, KATO-III Induce G1 arrest on MDA-MB-435, MCF-7, HT-29	Yoshimizu et al., 2004 Morley et al., 2007
	Apoptosis	-	Induce apoptosis on TMK-1, MKN-45, MKN-74, KATO-III cells	Yoshimizu et al., 2004
	Angiogenesis and metastasis	ERK-2 HIF1-α	Inhibit the activity (in silico study)	Puspita et al., 2008
	Estrogenic	-	-	-
	Co-chemotherapeutic	-	Increased cytotoxicity of doxorubicin on MCF-7 and T47D cells	Meiyanto et al., 2011
Hesperidin 	Antioxidant	-	Scavenge DPPH radicals, hydroxyl radicals, superoxide anion radicals, hydrogen peroxides, and possess reducing power	Al-Ashaal and El-Sheltawy, 2011
	Yi et al., 2008	ABC	ABC	ABC
	Supression of carcinogenesis	PI3K	Inhibit the activity (in silico study)	Hastuti et al., 2008
	Cell cycle regulation	CDK2	Inhibit the activity (in silico study)	Adina et al., 2008
	Apoptosis	p53	Upregulation on MCF-7 cells	CCRC, Unpublished data
	Angiogenesis and metastasis			
	Estrogenic	-	Decrease serum and hepatic lipid concentration, reduce osteoporosis of ovariectomized rats	Chiba et al., 2003
	Co-chemotherapeutic	PgP - Bcl-2 Bax	Inhibit the activity (in silico study) Increase cytotoxicity of doxorubicin on MCF-7 cells by modulating cell cycle and induce apoptosis Downregulation on HeLa cells Upregulation on HeLa cells	Adina et al., 2008 Hermawan et al., 2010 CCRC, unpublished data- Kusharyanti et al., 2011 Kusharyanti et al., 2011

Phytochemical and Chmical Structure	Chemopreventive Effect	Molecular Targets	Biological Effect	Ref.
Hesperetin 	Antioxidant	-	-	-
	Supression of carcinogenesis	-	-	-
	Cell cycle regulation	CDK2, CDK4, Cyclin D	Downregulation on MCF-7 Cells	Choi, 2007
	Apoptosis	Caspase-3	Activation on HL-60 cells	Chen et al., 2003
	Angiogenesis and metastasis	-	-	-
	Estrogenic	-	-	-
	Co-chemotherapeutic			
Nobiletin 	Antioxidant	-	-	-
	Supression of carcinogenesis	-	-	-
	Cell cycle regulation	-	-	-
	Apoptosis	Bcl-2 Bax	Downregulation on HeLa cells; THP-1 cells Upregulation on HeLa cells; THP-1 cells	Park et al., 2008; Larasati et al., 2011
	Angiogenesis and metastasis	-	-	-
	Estrogenic	-	-	-
	Co-chemotherapeutic	BCRP PgP - Bcl-2 Bax	Inhibited the activity on MCF-7 cells and NCI-H460 lung cancer cells Inhibited the activity on MCF-7/ADR cells Increase cytotoxicity of doxorubicin on MCF-7 and T47D cells Downregulation on HeLa cells Upregulation on HeLa cells	Zhang et al., 2004 Chung et al., 2005 Fitriasari et al., 2010 Meiyanto et al., 2011 Larasati et al., 2011
Naringin 	Antioxidant	LTC4	Inhibit the transport	Leslie et al., 2001
	Supression of carcinogenesis	PI3K PKC	Inhibit the activity (in silico study)	Hastuti et al., 2008 Pratiwi et al., 2008
	Cell cycle regulation	p21 ATP	Upregulation in 5637 bladder cancer cells inducing G1 arrest Bind on PI3K binding site	Kim et al., 2008 Hastuti et al., 2008
	Apoptosis	CDK2	Inhibit the activity (in silico study)	Adina et al., 2008
	Angiogenesis and metastasis	-	-	-
	Estrogenic	-	-	-
	Co-chemotherapeutic	PgP	Inhibit the activity (in silico study)	Adina et al., 2008

In an *in vitro* study tangeretin alone induced G1 arrest by increasing the expression of CDK inhibitors p37 and p21 in COLO 205 human colon carcinoma cells (Pan et al., 2002). This compound has been proven to inhibit the growth of estradiol-stimulated T47D cells (Van Slambrouck et al., 2005). Inhibition of MCF-7 cell proliferation was observed after 4 days treatment and G1 phase arrest after 24, 48, and 72 hours treatment, respectively (Morley et al., 2007).

Nobiletin has been observed to be cytotoxic by modulating cell cycle on TMK-1, MKN-45, MKN-74, and KATO-III human gastric carcinoma cells (Yoshimizu et al., 2004). Another study showed that the compound induced G1 phase arrest on MDA-MB-435, MCF-7, and HT-29 cells (Morley et al., 2007).

Hesperetin shows anticancer activity on MCF-7 cells through cell accumulation at G1 phase by inhibiting the expression of CDK2, CDK4 and Cyclin D; increasing the expression of p21 and p27; and increasing the binding of CDK4 and p21, but not p27 or p57 (Choi, 2007). Whilst

Naringenin inhibited MDA-MB-435 cells proliferation (Kanno et al., 2005) and showed antiestrogenic activity on ERα+ cells (Totta et al., 2004). Naringin, a glycoside form of naringenin, inhibited proliferation 5637 bladder cancer cells and induced G1 arrest by upregulation of p21 (Kim et al., 2008). Naringin also inhibited cell survival pathway by binding ATP on PI3K binding site (Hastuti et al., 2008).

Effect on Apoptosis

The balance between cell death and growth are important in the development and maintenance of body functions in multicellular organisms (Broker et al., 2005). The process of cell death also serves to repair damaged tissue and release cells that may be harmful (Hanahan and Weinberg, 2011). Disturbances in the balance between death and cell growth can cause pathological disorders such as disturbances in embryogenesis, degenerative neurological disease, and cancer development. Therefore, the balance between cell death and growth are strictly controlled and the presence of interference can be eliminated through a process called programmed cell death (Broker et al., 2005).

Apoptosis, among others, characterized by cell shrinkage, bebbling its plasma membrane, chromatin condensation, formation of apoptotic bodies and DNA fragmentation (Ward et al., 2008). This process requires ATP and the synthesis of certain proteins without an accompanying inflammatory response (Ward et al., 2008). Apoptosis may occur through two main pathways of extrinsic and intrinsic pathways. Extrinsic pathway involves activation of death receptors by ligand binding resulting in activation of caspase 8, whereas the intrinsic pathway occurs through mitochondrial pathway (Ward et al., 2008).

Tangeretin has been observed to have the lowest IC_{50} value on COLO 205 human colon carcinoma cells by triggering apoptosis and increasing p53 expression, compared with the other flavonoid compounds, such as apigenin, kaempferol, myricetin, quercetin, luteolin, nobiletin, and rutin (Pan et al., 2002). Tangeretin was also able to induce apoptosis on HL-60 human promyelocytic leukemia cells (Hirano et al., 1995). While nobiletin, another flavonoid obtained from *C. reticulata* peels, has been observed to be cytotoxic not only through modulating cell cycle, but also by inducing apoptosis on TMK-1, MKN-45, MKN-74, and KATO-III human gastric carcinoma cells (Yoshimizu et al., 2004).

Biological activites that have been observed *in vitro* were then followed by in vivo studies. Ethanolic extract of *C. reticulata* peels showed antiproliferative effect on rats' mammary and liver carcinogenesis model in a dose dependent manner. The extract induced apoptosis on liver cells, but not on mammary cells, and increased p53 expressions on both tissues (Pratomo et al., 2008).

The concentration of 40 and 80 μM hesperetin shows cytotoxic activity and could activate caspase 3 on HL-60 cells stronger than hesperidin did. At the same concentration, hesperetin induces apoptosis on HL 60 cells, while hesperidin does not. Rutinoside group at C-7 causes the reduction of apoptotic induction on HL 60 cells by hesperidin (Chen et al., 2003). Naringenin induced apoptosis via p53 independent pathway on KATOIII and MKN-7 gastric cancer cells and HepG2, Hep3B and Huh7 liver cancer cells and also via intrinsic pathway by downregulation of Bcl2 and upregulation of Bax, caspase-3 activation and PARP cleavage on THP-1 leukemia cells (Kanno et al., 2005; Park et al., 2008).

Effect on Angiogenesis and Metastasis

Cancer cells can stimulate angiogenesis. Angiogenesis is the growth of new blood vessels around the cancer tissue to meet the needs of nutrients and oxygen to cells. If the requirements have been met with good cells, easily cancer cells can invade other tissues. Cancer cells have the ability to invade and spread throughout the body tissues. The ability of cancer cells to invade and spread to other tissues occurs through blood vessels and lymph vessels are called to the stage of metastasis (Hanahan & Weinberg, 2011).

C. reticulata extract possesses angiogenic activity by increasing VEGF expression on WiDr colorectal cancer cells (Puspita et al., 2008). In contrast, anti-angiogenic effect on bFGF-induced chicken CAM's embryo by inhibiting new blood vessels formation both macroscopically and microscopically, and also reducing the number of macrophage cells were observed (Chrisnanto et al., 2008).

Several *in silico* studies have been conducted to observe tangeretin and nobiletin's binding on certain proteins. To predict their mechanism of action in inducing angiogenesis, tangeretin and nobiletin are docked to the proteins ERK-2 and HIF1-α, respectively. Both molecules does not bind ERK-2 and HIF1-α as strong as ATP does. Despite of those data, *in vivo* study showed that still, those phytochemicals are potential to be developed as therapeutic agent for heart revascularization and placenta formation in pregnancy (Puspita et al., 2008).

Estrogenic Effects of Citrus Flavonoids

Estrogen plays an important role in the development and differentiation of breast and endometrial cells, thus may have certain effects in the growth of cancer (Glaeser et al., 2006). However, estrogen also plays an important role in maintaining bone density, cholesterol level, and reproductive tissues (Jordan, 2004). Those effects are mediated by specific receptor, that is estrogen receptor (ER). The binding of estrogens to ER plays an important role in the occurrence of estrogenic effect (Rollerova & Urbancikova, 2000). The binding triggers or inhibits the transcription of certain genes being responsive to estrogens. Besides, estrogen-ER complex may interact with transcription factors, such as SP-1 (Meyer et al., 2006). So, ER targeting could be an approach of both chemoprevention and cancer targeted therapy (Manni et al., 2011). Compounds possessing similar effect to estrogen is called to have estrogenic effect, and numerous biocompounds have been shown to be active as phytoestrogens.

Interactions of estrogen and ER affect the expression of HDL and LDL receptors, resulting in the increasing

of HDL and decreasing of LDL level, thus play a role in cholesterol modulation (Meyer et al., 2006), whilst estrogen's role in maintaining bone density occurs through its ability to increase the activity of osteoblast and suppress the activity of osteoclast. The binding of estrogen to ER also may activate certain transcription factors, such as c-Myc, that later transcripts genes resulting in the activation of cell cycle.

Citrus reticulata ethanolic extract was potential to be developed as phytoestrogen. The extract shows proliferative effect on WiDr cells in low concentrations, not by affecting COX-2 expression (Ardiani et al., 2008). Forty-eight-hours single treatment of *C. reticulata* ethanolic extract on MCF-7 cells tend to show proliferative effect in a dose dependent manner, while combination with doxorubicin might suppress its proliferative effect (Yunas et al., 2007).

C. reticulata peels ethanolic extract with the dosage of 500 mg/kgBW and 1000 mg/kgBW could induce rats' mammary gland development by increasing c-Myc expression (Supriyati et al., 2008). The extract could increase uterus' volume of Sprague-Dawley rats by increasing c-Myc expression (Adelina et al., 2008a). Besides, *C. reticulata* peels extract increased bone density and improved blood cholesterol profile in dose dependent manner (Adelina et al., 2008b), whilst hesperidin decreased concentration of serum and hepatic lipid and reduced osteoporosis of ovariectomized rats (Chiba et al., 2003).

Citrus Flavonoids as Potential Cancer Co-Chemotherapeutic Agents

The phenomenon of resistance of breast cancer cells will decrease the efficacy of chemotherapeutic agents, so that research on breast cancer is directed at the development of the companion agent in combination chemotherapy. Combination with a chemopreventive agent can be an alternative option to overcome the problem of resistance of cancer cells and increase the effectiveness of chemotherapeutic agents (Fimognari et al., 2006). The combination is expected to be able to reduce drug toxicity to normal tissue, making it more effective in fighting cancer cells (Sharma et al., 2004; Tyagi et al., 2004).

Ethanolic extract of Citrus aurantiifolia peels inhibited MCF-7 cells growth (IC50 59 g/ml) and increased the sensitivity of MCF-7 breast cancer cells againts doxorubicin (Adina et al., 2008) by apoptosis induction and cell cycle modulation (CCRC, unpublished data). Hesperidin, a flavonoid in *C. aurantiifolia* has been studied in combination with doxorubicin to increased cytotoxic effect in concentration of 200 nM doxorubicin and 100 μM hesperidin (Hermawan et al., 2010), modulated cell cycle and induced apoptosis of MCF-7 cells (CCRC, unpublished data). Hesperetin, aglycone of hesperidin, showed stronger cytotoxic activity than hesperidin because its' less polarity makes it easier to penetrate the cell membrane (Chen et al., 2003).

In combination, tangeretin synergistically increases the cytotoxic effect of doxorubicin by inducing cells' death and arresting cell cycle's phase on MCF-7 and T47D breast cancer cells (Meiyanto et al., 2011[a]). Observation showed that nobiletin increased doxorubicin's cytotoxic activity on MCF-7 cells, but not on T47D cells (Meiyanto et al., 2011[b]).

Previous study showed that hesperidin synergistically increased doxorubicin cytotoxic activity on HeLa cells by downregulation of Bcl-2 and upregulation of Bax (Kusharyanti et al., 2011). Hesperetin is a molecule having similar structure with Pgp inhibitor. Thus, the compound is expected to inhibit Pgp overexpression and could overcome the resistance problem. Based on these researches, the potential of hesperetin as an anticancer agent or combinational agent in reducing the resistance of cancer cells with specific target has to be revealed.

Naringenin decreased *efflux* of daunomycin on MCF-7/ADR cells by inhibition of PgP (Chung et al., 2005). Naringenin also increased accumulation of mitoxantrone on MCF-7 cells and NCI-H460 lung cancer cells by inhibition of breast cancer resistance protein (BCRP) (Zhang et al., 2004). Naringenin also increased cytotoxicity of doxorubicin on MCF-7 cells and T47D cells (Fitriasari et al., 2010; Junedi et al., 2010) via apoptosis induction. Previous study showed that naringenin synergistically increased doxorubicin cytotoxic activity on HeLa cells by downregulation of Bcl-2 and upregulation of Bax (Larasati et al., 2011). Those data shows that citrus flavonoids are promising to be developed as co-chemotherapeutic agent in cancer therapy.

Citrus as a Model of Prospective Natural Product in Cancer Chemoprevention and Cochemotherapy

Cancer Chemoprevention Research Center (CCRC), Faculty of Pharmacy Universitas Gadjah Mada has explored several agents possessing biological activity related to cell growth and differentiation, either in the form of synthetic compounds or plant extracts. In this paper, at least two members of citrus family e.g *Citrus aurantiifolia* and *Citrus reticulata* has been discussed. Those could be a very valuable information to be analyzed further as the source of information for future studies regarding to the treatment and prevention of cancer. Besides, further and comprehensive studies on targeted therapy let us conduct a molecular approach in finding the scientific basis of natural products application (as traditional medicine) both solely and in combination with existing established drug, clinically.

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