Phytochemicals with direct and indirect anti-angiogenic properties against various cancer types focusing on their mechanism of action

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Abstract

Cancer remains the second leading cause of death despite continuous efforts that have been spent on cancer treatment. The escalating cases are due to several challenges in cancer therapy, including the non-selective toxicity of chemotherapies and chemoresistance. Therefore, alternative strategies are developed to prevent, reverse or delay the carcinogenesis process. One of these strategies is to inhibit or control angiogenesis, a process of forming new blood vessels from a pre-existing vessel. Several clinically approved anti-angiogenics showed evidence to suppress tumour growth and aggressiveness. However, some patients could not respond well to these therapies as expected due to cancer relapse or their side effects. Nowadays, phytochemicals have been receiving special attention in developing new molecules that can inhibit angiogenesis. This application is due to their pleiotropic behaviours, where phytochemicals could have multiple mechanisms affecting multiple signaling pathways, such as cell growth, apoptosis, and cell survival, in addition to their perceived safety over synthetic compounds. In this article, some phytochemicals are highlighted by referring to their mechanisms of action as anti-angiogenics. Based on the literature, most phytochemicals indirectly exert their anti-angiogenic effect; therefore, more attention should be pointed to the direct anti-angiogenic effect. In summary, this review described angiogenesis targets for some phytochemicals, providing helpful information for developing new selective anti-angiogenic therapy that can be used in combination with other chemotherapeutics or as chemopreventive agents.

Key Words: cancer, phytochemicals, direct anti-angiogenesis, indirect anti-angiogenesis

Significance | This review shows phytochemicals and their use in combination with chemotherapies or as chemoprevention.

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Introduction

Cancer and Angiogenesis

Cancer is the second leading cause of death globally (Jemal *et al.*, 2011). Cancer cases are expected to increase globally from 16.8 million in 2017 to 21.7 million by 2030 due to increased risk factors (Fidler, Soerjomataram and Bray, 2016). The non-specific toxicity of chemotherapeutics and the development of chemoresistance remain the main obstacles against cancer recovery and survival. Therefore, new strategies are necessary to prevent, reverse or delay the carcinogenesis process (Shankar *et al.*, 2017). It is hypothesized that about 80% of cancer patients would require alternative treatments, especially by applying pleiotropic compounds that act on different molecular targets to produce the synergistic effect (Banudevi, Swaminathan and Maheswari, 2015). One of the strategies is to modulate an important cancer hallmark which is angiogenesis.

Angiogenesis is defined as forming a new blood vessel from the

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pre-existing vessel that contributes to many physiological and pathological conditions (Jackson *et al.*, 1997; Carmeliet and Jain, 2000; Costa, Incio and Soares, 2007; Wälchli *et al.*, 2015). Physiologically, there is a strict balance between pro-angiogenic and anti-angiogenic functions to regulate the proliferation and angiogenesis of endothelial cells (ECs) (Ferrara, 2010). Induction of the angiogenesis process represents a hallmark in several inflammatory diseases and cancers (Carmeliet and Jain, 2000). Tumour angiogenesis provides the tumour with oxygen and nutrients, ultimately leading to subsequent tumour growth (Carmeliet and Jain, 2000; Ferrara, 2010). However, there is an increased tendency to suppress this process in patients diagnosed with cancer or who possess a high risk for cancer, leading to establishing the term 'angio-prevention therapy' (Albini *et al.*, 2012).

At present, most of the clinically approved anti-angiogenic therapies target the vascular endothelial growth factor (VEGF) pathway. However, due to the cancer relapse and side effects of the therapies, these anti-angiogenic therapies are ineffective against all patients (Vasudev and Reynolds, 2014). To overcome this limitation, new candidates with a different mechanism of action against angiogenesis are being investigated. In addition, special research attention was given to the natural compounds that possess anti-angiogenic properties but have minimal or no side effects compared to synthetic compounds (Duthie *et al.*, 2006).

Angiogenesis is a normal process that happens during embryonic development and wound healing. On the other hand, this process has an essential role in the malignant transformation of tumour growth. During the uncontrolled division of cancer cells, the tumour cannot grow without the additional supplementation of oxygen and nutrients. Therefore, angiogenesis has become a critical step for the metastatic spread of cancer, whereby cancer cells can split from the original tumour to enter blood vessels or lymphatic vessels and finally move to other sites to implant and start a secondary tumour (Rufino-Palomares *et al.*, 2015).

The anti-angiogenic therapy suggests that radiation and chemotherapy can act more efficiently in the presence of a stable microvascular environment surrounding the tumour than a highly diffusible unstable microvascular environment (Rufino-Palomares *et al.*, 2015).

Importance of phytochemicals

Phytochemicals are biologically active compounds found in fruits, vegetables and grains. They are generally classified as carotenoids, polyphenols, phenolic acids, indoles and compounds with high sulfur content (Liu, 2004). These phytochemical compounds, especially those extracted from traditional herbs, have gained specific research and commercial focus due to their safety, efficacy and potency for cancer prevention (Löhnert *et al.*, 2014; Hu *et al.*, 2016). These phytochemicals are proven to help treat or prevent cancer due to their pleiotropic function against every step in carcinogeneses such as cell cycle control, apoptosis and cell death control, angiogenesis, metastasis and genetic modulation. However, the application of phytochemicals could worsen the disease and cause other side effects if suitable regulations are not being complied (González-Vallinas *et al.*, 2013).

Roles of phytochemicals in the chemoprevention strategy

Cancer prevention is generally arranged into four strategies, covering lifestyle changing, early detection, assessment of genetic risks and chemoprevention. Among these strategies, chemoprevention which is gaining research popularity can reduce cancer risk by using natural or synthetic compounds that could suppress, reverse or prevent premalignant or malignant tumours (Batra and Sharma, 2013). These actions are possible by scavenging free radicals, targeting angiogenesis, pro-apoptotic effects, cell cycle control and initiation of DNA repair. The molecular targets for chemoprevention properties include vital factors such as nuclear factor-kappa (NF-k), activator protein-1, cell survival Akt, tumour suppressor p53, growth factors, tumour necrosis factor (TNF), signal transducer and activator of transcription (STAT), cyclooxygenase-2 (COX-2), lipooxygenases, inducible nitric oxide synthase (iNOS), and mitogen-activated protein kinase (MAPK) (Aggarwal and Shishodia, 2006; Khuda-Bukhsh, Das and Saha, 2014; Bolhassani, 2015).

Chemoprevention can be divided into three phases: primary chemoprevention for patients at high risk to develop cancer, secondary chemoprevention for patients with premalignant lesions, and tertiary chemoprevention targeted to prevent cancer recurrence (Landis-Piwowar and Iyer, 2015). The advantage of phytochemicals in chemoprevention is their ability to prevent different stages from the start of cancer disease and effective in covering all the major stages of cancer progression (Lluria-Prevatt and Alberts, 2008; Hanahan and Weinberg, 2017)

Phytochemicals have moieties that selectively regulate DNA damage, cell proliferation, inhibit angiogenesis and induce apoptosis and autophagy (Mukhtar *et al.*, 2012). About one-third of cancer cases are preventable by increasing the daily intake of fruits and vegetables (Khan, Afaq and Mukhtar, 2008). As a result, the World Cancer Research Fund (WCRF) and American Institute for Cancer Research (AICR) have recommended individual to have more consumption of fruits and vegetables to help in preventing or decreasing the probability of cancer development and progression (Key *et al.*, 2004).

There are several examples of phytochemicals that are useful for chemoprevention. For example, apigenin, a type of flavonoid, possesses anti-inflammatory, antioxidant and anti-carcinogenesis effects and chemoprevention properties (Kim, Kim and Sung, 2003). Another example is curcumin, the active ingredient of turmeric that has been recommended to be used as a

chemoprevention agent for colorectal cancer (Goel, Kunnumakkara and Aggarwal, 2008; Prasad, Tyagi and Aggarwal, 2014). Besides, ginsenoside found in Panax Ginseng could also inhibit cancer by inducing pro-apoptotic pathways and modulation of the immune system. Last but not least, licorice, which contains flavonoids, was the chemopreventive agent with anti-angiogenic properties (Li *et al.*, 2015; Chen *et al.*, 2016; Wang, Anderson and Yuan, 2016).

Angiogenesis regulators

Many pro-angiogenic factors bind to their corresponding receptors of targeted genes/pathways, resulting in physiological and pathological angiogenesis control. VEGF's most crucial regulator, with its target receptor in the vascular endothelial growth factor receptor (VEGFR) pathway. Besides that, there are many other pro-angiogenic growth factors such as angiopoietin and its receptor (Ang-2/Tie2), platelet-derived growth factor (PDGF/PDGFR), basal fibroblast growth factor (bFGF/FGFR) pathways, transforming growth factor (TGF-β), angiogenin, and interleukin-8 (IL-8). All these growth factors are secreted by cancer tissue to stimulate, proliferate and induce the migration of EC towards the tumour, subsequently cause an excessive build of new blood vessels (Kerbel, 2008).

Metalloproteinases (MMPs) also plays an essential role in angiogenesis and metastasis by dissolving the basement membrane and extracellular matrix (Koskensalo *et al.*, 2011). The first step in angiogenesis is vasodilation and increased permeability through the release of VEGF. This step is accompanied by losing pericyte covering mediated by the Ang-2/Tie2 pathway (Bergers and Benjamin, 2003; Fagiani and Christofori, 2013). During this time, the release of MMPs and heparanases dissolve the basement membrane and generate more pro-angiogenic factors (Vlodavsky and Friedmann, 2001). As a result, an increased amount of pro-angiogenic factors would drive the migration of EC, forming tube-like structures and subsequently encouraging the formation of new blood vessels (Bergers and Benjamin, 2003). These new blood vessels are sustained by recruiting other types of stromal cells, such as fibroblasts. In response to cancer cells growth factors, these blood vessels will promote angiogenesis and metastasis by secreting large amounts of MMPs and cytokines (Giannoni *et al.*, 2010).

Hypoxia is another critical step in angiogenesis. It turns on the angiogenesis switch and tips the balance by favouring the pro-angiogenic over the anti-angiogenic effects. This cellular event happens through the upregulation of transcriptional factor hypoxia-inducible factor (HIF-1α) (Pugh and Ratcliffe, 2003).

Anti-angiogenic therapy

Anti-angiogenic therapy can be classified into two categories: direct anti-angiogenics and indirect anti-angiogenics. Direct anti-angiogenics include inhibitors that target microvascular EC and prevent them from reacting to various pro-angiogenic stimuli. On the other hand, indirect anti-angiogenic exert their effect by interfering with pro-angiogenic communication between tumour and EC compartments. In addition, some tumours can produce different pro-angiogenic factors to escape the inhibitory effect of indirect anti-angiogenics; an example would be the upregulating release of bFGF instead of VEGF (Yoshiji, Harris and Thorgeirsson, 1997).

Direct anti-angiogenics

Anti-angiogenic therapy starts from the presence of natural angiogenesis inhibitors, such as angiostatin, endostatin, arrestin and other factors released from digestion of the extracellular matrix. They inhibit EC proliferation and migration even in the presence of angiogenic inducers due to interference with endothelial integrins or internal survival pathways (Kerbel and Folkman, 2002). Many strategies for direct anti-angiogenic therapies have been investigated. One of the strategies includes cell adhesion molecules targeted therapy that binds integrins on EC to suppress physical connections and signaling pathways such as migration, proliferation and survival (Nyberg, Xie and Kalluri, 2005). The compounds that target cadherins, a superfamily that controls cell-cell adhesions, are an example of direct anti-angiogenics.

Another strategy for direct anti-angiogenic therapies is by targeting Delta/Jagged-Notch pathway. The receptor Notch (1-4), their ligands Delta-like (Dll1, 3 and 4) and jagged 1, 2 play an essential role in the development of angiogenesis. DII4 was overexpressed in the endothelium of tumour blood vessels, but it was not expressed in normal blood vessels (Mailhos *et al.*, 2001). More interestingly, selective inhibition of Dll4 leads to a vascular network that is poorly formed and non-functional, and a significant decrease in tumour size was observed (Ridgway *et al.*, 2006). Moreover, a decrease in tumour size was observed in tumours that are resistant to the VEGF blockade.

Inhibition of Jagged 1 by using a soluble Notch receptor decoy was found to block angiogenesis in both *in vitro* and *in vivo* models and decrease tumour growth (Funahashi *et al.*, 2008).

In general, studies have revealed that targeting EC, which are genetically stable compared to the fast-dividing genetically-non stable cancer cells, is more efficient and decreases the incidence of developing drug resistance (Kerbel and Folkman, 2002; Rufino-Palomares *et al.*, 2015).

Indirect anti-angiogenics

Indirect anti-angiogenics are agents that prevent the expression or block the activity of angiogenic inducer proteins. Some examples of these agents are bevacizumab that neutralises the released VEGF, and sunitinib which blocks VEGFR1, 2 and 3. In addition, indirect anti-angiogenics also include oncogene targeted therapy

and conventional chemotherapeutic agents, where these therapies target the inflammatory process that releases factors that stimulate VEGF production.

Other pathways include inhibition of expression of HIF-1α as studies revealed that most solid malignancies express it in high quantities (Koukourakis *et al.*, 2001).

Phytochemicals with their mechanism of action as direct or indirect anti-angiogenic

Apigenin

Apigenin (4',5,7- trihydroxyflavone) is a major plant flavone derived from the *Apium* genus in the Apiaceae family. It is commonly found in parsley, chamomile, celery, vine spinach, and different vegetables and fruits (McKay and Blumberg, 2006). Apigenin has anti-inflammatory, antioxidant, anticancer properties and exhibits chemopreventive effects. The anticancer properties are linked to its antioxidant activity against oxidative stress, DNA damage, inhibition of angiogenesis and inflammation, cell growth suppression and induction of apoptosis and autophagy (Sung, Chung and Kim, 2016).

Moreover, apigenin has an anti-angiogenic effect against various solid malignancies, including breast, colon, gastric, pancreatic, lung and prostate cancers due to the inhibitory effects on several protein-tyrosine and serine kinases including MAPK, Akt, phosphatidylinositol 3-kinase (PI3K) and the cytokine-activated Janus kinase (JAK) kinases that will interrupt signalling pathways such as insulin-like growth factor (IGF), NF-kB, STAT, and p53 (Kim, 2003; Shukla and Gupta, 2010). In addition, apigenin downregulates the transcription of enzymes, growth factors or modulators involved in the angiogenesis process, such as MMP2, MMP9, VEGF and COX-2, which affect cell migration and invasion (Huang *et al.*, 2016). Furthermore, apigenin binds to HIF-1α, inhibiting VEGF and HIF-1α expressions in cancer cells (Liu *et al.*, 2005). Moreover, apigenin exerts a selective inhibitory effect on ECs proliferation and tube formation *in vitro* with no *in vivo* response (Engelmann *et al.*, 2002).

Abisilin

Abisilin is a naturally occurring terpenoid found in *Abies sibirica* coniferous trees of a *Pinaceae* family. This terpenoid has a beneficial effect to counteract angiogenesis. Moreover, it has anti-inflammatory, painkilling, anti-ageing and immunomodulatory properties. The chemoprevention effect of abisilin is linked to pro-apoptotic and cell cycle arrest properties *in vitro* and *in vivo* (Kudryavtseva *et al.*, 2016). Furthermore, the *in vitro* and *in vivo* studies on anti-angiogenic activity showed an inhibitory and reduction of neovascularisation in xenograft tumours, which were suspected to be caused by downregulation of VEGF and intracellular adhesion molecule-1 (ICAM-1) expression important as regulators of endothelial cell growth, migration and survival (Kevil *et al.*, 2004).

***Pracitrullus fistulosus* lectin protein (pflp)**

Pflp is a carbohydrate-binding protein present in the phloem exudates of the fruit sap of *Pracitrullus fistulosus* belonging to the family Cucurbitaceae (Cavada *et al.*, 2001). Generally, lectins have biological activities as cellular pathway modulators and carry anti-insect, antitumor, antibacterial and antinociceptive effects (Liu *et al.*, 2013; Nascimento *et al.*, 2016). Pflp has shown both apoptotic and cytotoxic properties against multiple cancer cell lines with minimal toxicity toward normal cell lines *in vitro*. Where tested *in vivo*, pflp could increases the life span of the *Ehrlich ascites* carcinoma (EAC) animal model by 18 days versus 47 days for the treated group. In addition, Pflp has anti-angiogenic properties represented by a decrease in blood vessels formation *in vivo* due to the downregulation of VEGF, MMP-2 and MMP-9 activities (Shivamadhu *et al.*, 2017).

Betulinic Acid (BA)

Betulinic acid (BA) is a pentacyclic triterpenoid present in many plants worldwide (Cichewicz and Kouzi, 2004) and exhibits antibacterial, antimalarial, antioxidant, anti-inflammatory, and antiviral activity properties. The antitumor effect of BA is mainly due to the induction of apoptosis (Fulda, 2008). Moreover, it was reported that BA has anti-angiogenic properties due to the activation of selective proteasome-dependent degradation of the transcription factor specificity protein SP1, SP2 and SP4 with downregulation of gene expression of VEGF, MMP9, and HIF-1α (Chintharlapalli *et al.*, 2007). Furthermore, other studies revealed that BA exerts an inhibitory effect on microRNA miR-27a, with the induction of its target ZBTB10 leading to the suppression of transcription factors SP1, SP2, SP3, and SP4 and finally contribute to the process of angiogenesis (Mertens-Talcott *et al.*, 2013).

Ursolic Acid (UA)

Ursolic acid (UA) is a ursane-type pentacyclic triterpenoid found in many medicinal plants and fruits (Liobikas *et al.*, 2011). UA has anticancer (Lin *et al.*, 2013), anti-inflammatory (Ku and Lin, 2013), hypoglycemic (Shanmugam *et al.*, 2013) and antioxidant effects (Liobikas *et al.*, 2011). The anticancer properties are due to the induction of apoptosis in many cancer cells, including prostate, colon and lymphoma cells (Sun *et al.*, 2006). The downregulation contributes the anti-angiogenic properties in the gene expression of VEGF, MMP9, HIF-1α and IL-8 (Lin *et al.*, 2010). However, UA has shown a pro-angiogenic effect through the upregulation of genes encoding E-selectin, ICAM-1, VEGF, bFGF and receptors (Kiran *et al.*, 2008).

Epigallocatechin gallate (EGCG)

EGCG is a polyphenol present in green tea with anticancer, anti-angiogenic, and metastasis inhibition properties. Inhibition of VEGF expression is the mechanism of EGCG that contributes to its anti-angiogenic property (Jung *et al.*, 2001).

Reservatrol

Reservatrol is a polyphenol that has been detected in at least 72 plant species. It has broad biological activities, covering antioxidant, apoptotic, anticarcinogenic and anti-infectious properties. In addition, it has been reported that reservatrol exhibits anti-angiogenic properties due to its effect in causing the downregulation of genes encoding VEGF, MMP9 and COX-2 (Harikumar *et al.*, 2010).

Scopoletin

Scopoletin is a coumarin-derived compound with anti-angiogenic properties, where it is isolated from the tobacco plant, *Nicotiana glauca*, from the Solanaceae family (Beh *et al.*, 2012). Scopoletin has many health-related bioactive activities, including hepatoprotective (Kang *et al.*, 1998), antioxidant (Shaw *et al.*, 2003) and spasmolytic properties (Oliveira *et al.*, 2001). In addition, Scopoletin arrests the cell cycle leading to the induction of apoptosis (Liu *et al.*, 2001). Research showed that Scopoletin has a selective growth inhibition mechanism against EC with minimal effect on other cells (Thani *et al.*, 2010; Tabana *et al.*, 2016). When tested *in vivo*, scopoletin show 94.7% inhibition for the tumour growth at the dose of 20 mg/kg applied on the HCT116 (i.e. human colon cancer cell line) xenograft animal model. The anti-angiogenic properties are related to the blocking effect on VEGF, bFGF2 and the extracellular-signal-regulated kinase (ERK1) signalling pathways (Tabana *et al.*, 2016).

The previous phytochemicals with their anti-angiogenic mechanism of action are summarised in Table 1.

CONCLUSION

Phytochemicals are an excellent choice to inhibit angiogenesis due to their expected safety and ability to target several steps in carcinogenesis, such as apoptosis and cell death control, angiogenesis, and metastasis. Unfortunately, only two compounds have proven direct anti-angiogenesis effects against ECs proliferation, migration or physical connections. The outcomes of this review would encourage the research community to conduct systematic or rational screening of phytochemicals or medical plants to discover natural anti-angiogenic compounds that will eventually be used in combination with chemotherapies or as chemoprevention.

Table 1. Phytochemicals with their mechanism of action as direct or indirect anti-angiogenic

Phytochemicals	Mechanism of actions	Direct or Indirect anti-angiogenic	References
Apigenin	-Inhibitory effect on MAPK, PI3K-Akt and JAK kinases. -Downregulating MMP2, MMP9, VEGF, HIF-1α and COX-2. -Selective inhibitory effect on EC proliferation and tube formation *in vitro* with no *in vivo* response.	Direct and Indirect	(Shukla and Gupta, 2010)-54).
Abisilin	-Downregulating ICAM-1 expression.	Indirect	(Kevil *et al.*, 2004)
pflp	-Downregulation of VEGF, MMP-2 and MMP-9 activities.	Indirect	(Shivamadhu *et al.*, 2017)
Betulinic Acid	-Suppression of the transcription factors SP1, SP2, SP3 and SP4. -Downregulating expression of VEGF, MMP9 and HIF-1α.	Indirect	(Chintharlapalli *et al.*, 2007) (Mertens-Talcott *et al.*, 2013)
Ursolic Acid	-Downregulating expression of VEGF, MMP9, HIF-1α and IL-8.	Indirect	(Lin *et al.*, 2010)
EGCG	- Downregulating expression of VEGF.	Indirect	(Jung *et al.*, 2001)
Reservatrol	-Downregulating VEGF, MMP9 and COX-2 expressions.	Indirect	(Harikumar *et al.*, 2010)
Scopoletin	-Blocking VEGF, FGF2 and ERK1 signalling pathways. -Selective growth inhibition against ECs.	Direct And	(Thani *et al.*, 2010), 80)

Author Contributions

All authors conceived the study. IAD drafted the manuscript, wrote the manuscript and prepared the table of findings. NAS, AMSAM, and JJ contributed to manuscript revisions. All authors approved the final version of the manuscript.

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Competing financial interests

The author(s) declare no competing financial interests.

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