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

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...
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 (Löhntert 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, Afacq 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 proangiogenic 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. Dll4 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 oncongene targeted therapy.
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).

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

### Apigenin

Apigenin 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 (Kevill et al., 2004).

Abisilin 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).

### 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 (Shivamadhuv 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 (Chincharlapalli 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 antinecancer (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 (Kirian 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).
Resveratrol
Resveratrol 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 resveratrol 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 spasmylytic 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*, scopoeletin 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

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<td>Apigenin</td>
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<td>Direct and Indirect</td>
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<td>Abisilin</td>
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<td>pflp</td>
<td>- Downregulation of VEGF, MMP-2 and MMP-9 activities.</td>
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<tr>
<td>Betulinic Acid</td>
<td>- Suppression of the transcription factors SP1, SP2, SP3 and SP4. &lt;br&gt; - Downregulating expression of VEGF, MMP9 and HIF-1α.</td>
<td>Indirect</td>
<td>(Chintharlapalli et al., 2007) (Mertens-Talcott et al., 2013)</td>
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<tr>
<td>Ursolic Acid</td>
<td>- Downregulating expression of VEGF, MMP9, HIF-1α and IL-8.</td>
<td>Indirect</td>
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<td>EGCG</td>
<td>- Downregulating expression of VEGF.</td>
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<td>Resveratrol</td>
<td>- Downregulating VEGF, MMP9 and COX-2 expressions.</td>
<td>Indirect</td>
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<td>Scopoletin</td>
<td>- Blocking VEGF, FGF2 and ERK1 signalling pathways. &lt;br&gt; - Selective growth inhibition against ECs.</td>
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Author Contributions
All authors conceived the study. IAD drafted the manuscript, wrote the manuscript and prepared the table of findings. NAS, AMSAM, and J contributed to manuscript revisions. All authors approved the final version of the manuscript.

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