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Review

Proangiogenic activity of plant extracts in accelerating wound healing — a new face of old phytomedicines

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Angiogenesis, the formation of new capillaries from pre-existing vascular network, plays an important role in physiological and pathological processes such as embryonic development, wound healing, and development of atherosclerosis. Extension of the circulatory network is also considered to be one the most important factors during cancerogenesis. Inhibition of angiogenesis may lead to inhibition of tumor growth whereas stimulation may improve wound healing. Research achievements suggest the use of plants and their extracts as potential therapeutic agents with pro- or antiangiogenic activity. Since the anticancer and antiangiogenic properties of many phytomedicines have been amply reviewed elsewhere this paper will focus on the treatment of vascular insufficiency in wound healing. Globally accepted herbal drugs are thought to be safe and effective, however, there is a need for more evidence-based confirmation in controlled and validated trials. Among the most frequently studied proangiogenic phytochemicals are ginsenosides from *Panax ginseng*, beta-sitosterol from *Aloe vera*, calycosin from *Radix Astragali*, and extracts from *Hippophae rhamnoides* L. and *Angelica sinensis*.

Keywords: wound healing, angiogenesis, phytomedicines, plant extracts

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INTRODUCTION

Wounds have affected humans since prehistoric times and the treatment and healing of wounds is an art as old as humanity (Robson *et al.*, 2001). Due to the increasing life expectancy coupled with a more modern way of life, wounds and particularly chronic wounds increasingly affect a growing number of elderly patients and seriously reduce their quality of life. Current estimates indicate that nearly 6 million people suffer from chronic wounds (Kumar *et al.*, 2007) causing great physiological and mental trauma. In the United States, chronic wounds cost the nation $20 billion to $25 billion a year, and acute or traumatic wounds add another $7 billion to $10 billion annually. Research on wound healing drugs is a rapidly developing area of modern biomedical sciences. The progress in this field has allowed the synthesis of large numbers of molecules associated with wound repair process. Delivery of exogenous growth factors in order to mimic the natural microenvironments of tissue formation and repair is believed to be therapeutically effective. The most important among the growth factors are recombinant human platelet-derived growth factor-BB and granulocyte colony-stimulating factor. The former has been approved by the U.S. Food and Drug Administration (FDA) for the treatment of neuropathic ulcers when there is adequate blood supply. However, recent analysis shows that their clinical efficacy is limited (Papanas & Maltezos; 2007). Despite finding new methods of stimulation of the wound repair process, wound care has returned to the roots of medicine and is embracing some of the remedies used millennia ago. Plant-derived natural products are significant as sources of medicinal agents and models for the design of new remedies. Such pharmaceuticals as morphine isolated from the opium poppy, salicylic acid from the bark of the willow tree or ephedrine from the Chinese herb mahuang are but a few examples of the many medicinally important substances (Fan *et al.*, 2006). Nowadays plant-derived compounds play an important role in drug development as exemplified by taxol and camptothecin (anticancer agents), artemisinin (the Chinese antimalarial drug), and forskolin (the East Indian Ayurvedic drug) (Balandrin *et al.*, 1993).

As plants are a source of many bioactive compounds and many plant ingredients are traditionally used to accelerate healing, scientists go back to traditional folk medicines as they are generally characterized by high acceptability and good toleration (Jagetia *et al.,* 2004).

The healing potential of phytomedicines is often associated with angiogenesis, which is a critical step of wound healing. It is the essential part of the repair process as it enables the nutrient supply to sustain cell metabolism, creates an intact delivery system, and facilitates the clearance of debris. Approximately 60% of the granulation tissue mass is composed of blood vessels which also supply the necessary oxygen to stimulate repair and vessel growth. Impaired wound healing may be a consequence of pathologic states associated with diabetes, im-

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Abbreviations: aFGF or FGF-1, acidic fibroblast growth factor; bFGF or FGF-2, basic fibroblast growth factor; CAM, chorioallantoic membrane; ECM, extracellular matrix; EGF, epidermal growth factor; eNOS, endothelial nitric oxide synthase; ER, estrogen receptor; ERK1/2, extracellular-signal-regulated kinases; FAK, focal adhesion kinase; FDA, food and drug administration; FGFR, fibroblast growth factor receptor; GR, glucocorticoid receptor; HIF-1, hypoxia-inducible factor 1; HUVEC, human umbilical vein endothelial cells; IGF, insulin-like growth factor; IGFR, insulin growth factor receptor; JNK, c-Jun N-terminal kinases; MAPKs, mitogen-activated protein kinases; MMPs, matrix metalloproteinases; PDGF, platelet-derived growth factor; PHF, poly-herbal formulation; PI3K, phosphatidylinositol 3-kinases; PKB, protein kinase B; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; SBT, seabuckthorn; TGF-α, transforming growth factor alfa; TGF-β, transforming growth factor beta; u-PA, urokinase-type plasminogen activator; VE-cadherin, vascular endothelial cadherin; VEGF, vascular endothelial growth factor; VEGFR-1, vascular endothelial growth factor receptor type-1 (Flt, Fms-like tyrosine kinase); VEGFR-2, vascular endothelial growth factor receptor type-2 (KDR/Flk-1; kinase-domain region/fetal liver kinase-1); vWF, von Willebrand factor.

mune disorders, ischemia, venous stasis, and in injuries such as burn, frostbite, and gunshot wounds. Patients with venous insufficiency associated for example with diabetes develop deep ulceration/wounds on the lower limbs (Pettet *et al.*, 1996). Proangiogenic factors present in wound fluid promote repair while antiangiogenic factors inhibit it. The healing efficacy seen in phytomedicine-treated wounds shows great promise although for most natural products no well-controlled scientific data are available.

WOUND HEALING AND ANGIOGENESIS

The Wound Healing Society defines wound healing as a "complex dynamic process that results in the restoration of anatomic continuity and function" (Strodtbeck, 2001). It involves interactions of extracellular matrix molecules, soluble mediators and various cells that cooperate to repair the injury. It also requires coordination of overlapping distinct cellular activities, involving phagocytosis, chemotaxis, mitogenesis, angiogenesis, and synthesis of collagen and other matrix components (Gurtner *et al.*, 2008). Wound repair is divided into four phases that overlap in time and space: haemostasis, inflammation, tissue formation (proliferative phase), and tissue remodeling. All these phases have specific contributions to blood vessel growth and remodeling (Schultz, 2007).

The haemostasis phase starts immediately after injury to prevent exsanguination and supply a matrix for invading cells. Within 24 hours platelets start to aggregate by binding to collagen that becomes exposed following rupture of the endothelial lining of vessels. The forming fibrin clot limits active bleeding and serves as a scaffolding for the recruitment of cells to the injured site. Additionally, it serves as a reservoir of the variety of growth factors and cytokines that are released as activated platelets degranulate. Insulin-like and epidermal growth factors (IGF, EGF), fibronectin, fibrinogen, histamine, platelet-derived growth factor (PDGF), serotonin and von Willebrand factor (vWF) act to control bleeding and limit the extent of injury. These molecules are also promoters of the wound healing cascade by activation and attraction of neutrophils, macrophages, endothelial cells and fibroblasts.

The inflammatory phase is characterized by influx of polymorphonuclear leukocytes (neutrophils). They are attracted to the wound within 24–36 h of injury by chemoattractive agents, like transforming growth factor beta (TGF-β). Neutrophils gathered in the wound environment phagocytose foreign material and bacteria as well as they are also a source of pro-inflammatory cytokines which may also serve as the earliest signals that activate local fibroblasts and keratinocytes. Subsequently macrophages, attracted by clotting factors, complement components, PDGF, TGF-β, leukotriene B_4, platelet factor IV, and elastin and collagen breakdown products appear in the wound and continue the process of phagocytosis. Besides, macrophages activate keratinocytes, fibroblasts and endothelial cells by releasing TGF-β, TGF-α, fibroblast growth factor (FGF), heparin-binding epidermal growth factors, and matrix metalloproteinases (MMPs). Macrophages also release PDGF and vascular endothelial growth factor (VEGF), which initiate the formation of granulation tissue and angiogenesis. Lymphocytes are the last cells entering the wound site during the late inflammatory phase (Strodtbeck, 2001; Velnar *et al.*, 2009).

During the proliferative phase, a granulation tissue (new stroma) is formed. Fibroblasts constitute the predominant cell type in granulation tissue. They start to proliferate and produce matrix components (hyaluronan, fibronectin, proteoglycans and type I and III procollagen), which are then deposited locally. Unwounded dermis contains about 80% type I collagen and 20% type III collagen, while new granulation tissue contains 30% to 40% type III collagen, which does not contribute to restoring tensile strength in the wound (Robson *et al.,* 2001). Fibroblasts change into their myofibroblast phenotype, which involves formation of pseudopodia capable of attaching to fibronectin and collagen in the extracellular matrix (ECM). This attachment starts the process of wound contraction leading to the approximation of the wound edges. In parallel to the formation of granulation tissue, increased proliferation and migration of keratinocytes takes place. During reepithelialization, basal keratinocytes undergo morphological changes required for their migration from the wound margin over the denuded area (Patel *et al.,* 2005). Epidermal cells secrete MMPs that break down collagen, and plasminogen activator which stimulates the production of plasmin. When the migration is complete, keratinocytes are stabilized by formation of firm attachments to the new basement membrane. When the skin surface is completely covered with new epidermal cells, the wound is considered healed (Strodtbeck, 2001).

At the beginning of the injury, there is no vascular supply to the wound centre and some uninjured vessels perfuse only the marginal viable tissue. The wound clot is then invaded by capillary sprouts from the surrounding edges which leads to composition of a microvascular network within several days. Angiogenesis (or neovascularization) is an essential part of the repair process as it enables nutrient supply to sustain cell metabolism and create an intact delivery system. Angiogenesis initiates with the relaxation of cell contacts and disruption of the outer pericyte layer. Hence, endothelial cells become free to migrate and proliferate to form new vessels. In order to open space for the proliferating cells, local degradation of the basement membrane and extracellular matrix is induced (Robson *et al.,* 2001). Angiogenesis is stimulated by growth factors and tissue hypoxia (Giordano & Johnson, 2001). A hypoxic wound environment is created following the closure of the wound surface by fibrin clot. The hypoxic conditions are thought to induce macrophages to secrete angiogenetic factors such as basic fibroblast growth factor (bFGF or FGF-2) and acidic FGF (aFGF or FGF-1) that are released immediately after cell disruption (Gurtner *et al.*, 2008). Also the production and release of PDGF and VEGF at the wound site is stimulated by hypoxia (Falanga, 2005). The shortage of oxygen causes an increase in the intracellular concentration of the active form of a gene regulatory protein called hypoxia-inducible factor 1 (HIF-1). HIF-1 binds to the hypoxia response element in the VEGF gene promoter region stimulating its transcription (Tsuzuki *et al.*, 2000). VEGF up-regulation can be also caused by ischemia and several cytokines or growth factors including EGF, TGF-β, keratinocyte growth factor 1, and others (reviewed in Ferrara, 1999). However, VEGF is considered to be the predominant and the most effective angiogenic mediator in human cutaneous wounds (Khanna *et al.*, 2001). VEGF acts by binding to two VEGF receptors, VEGFR-1 (Flt, Fms-like tyrosine kinase) and VEGFR-2 (KDR, kinase insert domain-containing receptor and its murine homolog, Flk-1). Activation of VEGFR-2 is connected with a mechanism dependent on the formation of a multi-protein complex that includes

VEGFR-2, phosphatidylinositol 3-kinases (PI3K) and the adherens junction proteins VE-cadherin and β-catenin (Carmeliet *et al.*, 2000). VEGF binding to cognate receptors on endothelial cells initiates autophosphorylation of VEGFR-2 that is followed by the activation of diverse angiogenesis-related enzymes such as mitogen-activated protein (MAP) and Akt/protein kinases B (PKB) to induce cell migration (Matsumoto & Claesson-Welsh, 2001). Furthermore, VEGF influences endothelial cells through the PI3K-Akt-eNOS signaling cascade, which is responsible for various biological activities in angiogenesis (Chen *et al.*, 2005). VEGF also induces angiogenesis by stimulating reactive oxygen species (ROS) production. ROS cause the oxidation of critical cysteine residues in protein tyrosine phosphatases such as SHP-2 thereby deactivating them (Chiarugi & Cirri, 2003). Besides, VEGF stimulates expression of urokinase-type activator, tissue-type plasminogen activator, plasmin, and matrix-degrading metalloproteinases capable of digesting basal lamina components (Hoeben *et al.*, 2004; Stetler-Stevenson, 2008). Endothelial cells produce four matrix metalloproteinases, MMP-1, MMP-2, MMP-9, and MT-1-MMP secreted as zymogens. Degradation of the extracellular matrix by MMPs and other extracellular enzymes (heparinases and plasmin) releases growth factors bound to the ECM (e.g., VEGF, FGF, PDGF, TGF-β) (Park *et al.*, 1993). Moreover, matrix metalloproteinases regulate VEGF bioavailability through intramolecular processing, and a subset of MMPs can cleave matrix-bound isoforms of VEGF, releasing soluble fragments. The matrix-bound VEGF and free VEGF provide different signaling outcomes although, in the case of endothelial cells, they both act through VEGFR-2 (Lee *et al.*, 2005).

VEGF is not the only growth factor inducing angiogenesis. Numerous growth factors such as angiopoietin-1/2, PDGF, FGF, and TGF-β are involved in the control of various aspects of angiogenesis (Fig. 1). The first identified proangiogenic molecule was bFGF. FGFs are strong mitogens not only for fibroblasts but also for vascular endothelial cells and smooth muscle cells. The biological effects of FGFs are mediated by four structurally related receptor tyrosine kinases, FGFR-1, -2, -3, and -4 (Cross & Claesson-Welsh, 2001). Angiopoietin-1/2 induces cell migration by signaling through Tie2 receptor whereas PDGF is required for vessel wall differentiation by recruiting pericytes to cover the outer surface of newly formed vessels. TGF-β enhances synthesis of matrix metalloproteinases such as collagenases, gelatinases and stromelysins that are needed to degrade the underlying extracellular matrix (Krump-Konvalinkova *et al.,* 2005). MMPs degrade collagen and other extracellular matrix elements, and through breaking down the basement membrane barrier, enable endothelial cells to migrate from pre-existing vessels towards angiogenic stimuli and to proliferate. The migration of endothelial cells is also facilitated by interactions between adhesion molecules, located on their surface (integrin $\alpha_v\beta_3$, α_2v, E-selectin), and specific components of the extracellular matrix (vitronectin, fibronectin, laminin, vWF). It has been reported that $\alpha_v\beta_3$ can bind MMP-2, enabling endothelial cells to degrade and remodel the ECM during their invasion (Eliceiri & Cheresh, 1999). The $\alpha_v\beta_3$ receptor is highly expressed on capillary sprouts that invade the fibrin clot. It has been shown that up-regulation of $\alpha_v\beta_3$ integrin on endothelial cells is mediated by nitric oxide (NO) which also stimulates endothelial cell podokinesis and increases dissolution of the extracellular matrix *via* the basic fibroblast growth factor–induced up-regulation of urokinase-type plasminogen activator. VEGF up-regulates expression of endothelial nitric oxide synthase (eNOS) mRNA providing a mechanism for prolonged VEGF-induced NO production (Cooke & Losordo, 2002).

Remodeling constitutes the final stage of wound healing, where granulation tissue is converted into mature connective tissue and/or scar. The vascularity and cellularity of the wound decrease while the extracellular matrix is reshaped by cross-linking of collagen (Parks, 1999). Although the new collagen increases the tensile strength of the wound, scar tissue can only be 80% as strong as unwounded skin (Tyrone *et al.*, 2000). During this phase, fibronectin gradually disappears and hyaluronic acid and other glycosaminoglycans are replaced by proteoglycans. This process is regulated by PDGF, TGF-β, FGF and many other factors. Subsequent wound healing is accompanied by elimination of fibroblasts and macrophages by

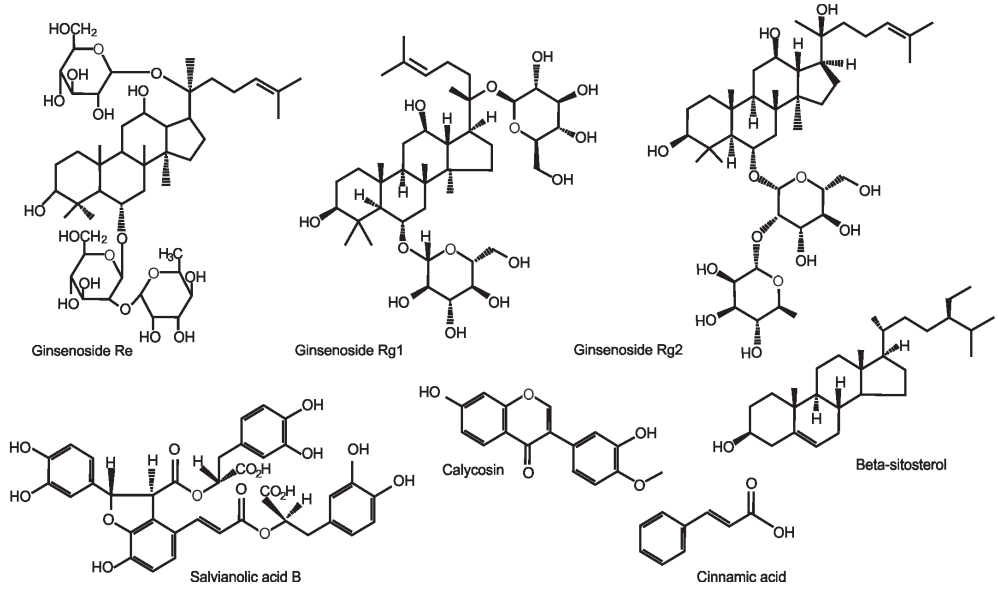

Figure 1. Proangiogenic compounds isolated from plants.

apoptosis. The growth of capillaries stops with time, the healing area is no longer supplied with blood at an increased rate, and the metabolic activities at the wound site decrease. The wound healing process culminates in a fully matured scar with a decreased number of cells and blood vessels (Velnar *et al*., 2009).

PLANT-DERIVED STIMULATORS OF ANGIOGENESIS

Research on wound healing drugs is a developing area in modern biomedical sciences and the search for compounds derived from plants constitutes a significant part of such studies. The use of plants and their extracts has its roots firmly embedded in the ancient civilizations of the East and Africa as well as those of Native American and Native South American cultures. More than 50% of all drugs in clinical use having a natural product origin show the importance of plants as a source of therapeutic agents (Balandrin *et al*., 1993). The stimulation of wound healing with herbal products is generally characterized by its high acceptability and good toleration (Jagetia *et al*., 2003). However, more hard data are necessary for a majority of proangiogenic plant-derived products, especially that most studies concern their antiangiogenic properties (Dulak, 2005). Although many natural products have been claimed to have healing effects, most do not have well-controlled scientific data. They need to be characterized in respect to the active chemical compounds, elucidation of the molecular mechanisms of their actions, demonstration of the real efficacy by *in vivo* studies and, finally, demonstration of their safety and effectiveness in clinical trials. A number of plants and their extracts are being investigated at present in this direction. Natural agents induce healing by multiple mechanisms, and in this review, medicinal plants as a source of novel angiomodulators stimulating angiogenesis are discussed. The primary challenge for wound healing improvement is to develop a vascular supply that can support the metabolic needs of the regenerated tissues. Products derived from a wide range of plants discussed below are known to have proangiogenic activities.

Panax ginseng

Panax ginseng (*Araliaceae* family) has been the most widely used herbal medicine in Eastern Asia for more than 2000 years (Attele *et al.,* 1999). Ginseng has multiple pharmacological actions for treating cardiovascular diseases, rheumatoid arthritis, and in the repair of intractable skin ulcers of patients with diabetes mellitus (Huang *et al*., 2005, Morisaki *et al*., 1995). Until now, twelve species have been identified in the genus *Panax* (Yue *et al*., 2007). Among them, *Panax ginseng* C. A. Meyer (Chinese, Asian or Korean ginseng) cultivated in China, Korea, Japan, Russia, and the US, *P. quinquefolium* L (American ginseng), grown in southern Canada and the US, and *P. notoginseng (*Sanqi ginseng), cultivated in the Yunnan and Guangxi provinces in China are the most extensively studied. The primary active components of ginseng are saponins including more than 40 identified ginsenosides classified as panaxdiols or panaxtriols (Lu *et al*., 2009). The ginseng root contains 2–3% ginsenosides of which Rb1 and Rg1 are the most abundant (Sengupta *et al*., 2004). The mass-spectroscopic compositional analysis performed by Sengupta *et al.* has revealed that Rg1 and Rb1 are present in all investigated extracts, however, each extract displays distinct ginsenoside composition, especially in the ratio between the two. American ginseng has a higher content of ginsenosides than other ginseng species. Extracts from American and Chinese ginseng have a predominance of Rb1 in contrast to Sanqi ginseng with its predominant ginsenoside Rg1. The ratio of Rg1 and Rb1 is especially important in the context of their opposite effects on angiogenesis: the dominance of Rg1 leads to angiogenesis, whereas Rb1 dominance exerts an opposing effect. Hong and coworkers (2009) have determined the angiogenic activity of total saponins from *P. notoginseng* (containing 11 saponins). Using human umbilical vein endothelial cells (HUVEC) as an *in vitro* model for studies on angiogenesis they have shown that the extract stimulates proliferation and tube formation by the cells. *P. notoginseng* saponins also stimulate VEGF and KDR/Flk-1 mRNA expression. The results obtained by Hong and coworkers suggest that the proangiogenic effects involve the VEGF-KDR/Flk-1 and PI3K-Akt-eNOS signaling pathways. It has also been demonstrated that Rg1 alone increases cell proliferation, migration and tube formation, and its angiogenic activities have been confirmed *in vivo* (Liang *et al*., 2005; Yue *et al*., 2005). Sengupta and coworkers (2004) have revealed that Rg1 (Fig. 2) promotes functional neovascularization through the expression of eNOS and the PI3K/Akt pathway. In contrast, Rb1 inhibits invasion of endothelial cells — the earliest step in angiogenesis. In that study neither Rg1 nor Rb1 had any effect on the mitogen-activated protein kinase pathway.

Previously, Rg1 had been demonstrated to trigger transcriptional activation of a glucocorticoid responsive element-containing reporter gene, suggesting that Rg1 can activate glucocorticoid receptor (GR) (Lee *et al*., 1997). Further studies by Leung *et al.* (2006) elucidated the effects of Rg1 on the eNOS system. They have shown in HUVECs that Rg1, being a functional ligand of GR, increases phosphorylation and the activities of PI3K, Akt and eNOS, leading to increased NO production. Additionally, Rg1 ginsenoside down-regulates the expression of adhesion molecules such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin (Lü *et al*., 2004), and up-regulates a set of genes related to cell adhesion, migration and cytoskeleton such as RhoA, RhoB, IQ-motif-containing GTPase activating protein 1, calmodulin, Vav2 and laminin-α4 (Yue *et al*., 2005). Ma *et al.* (2006) have also reported that in TNF-α-stimulated HUVECs the expression level of mitogen-activated protein kinase kinase kinase 3, reticulocalbin, phosphoglycerate mutase, 6-phosphogluconolactonase, zinc finger protein, nephritis strain-associated protein 1, and recombination-activating protein is increased, while that of eNOS and mineralocorticoid receptor is decreased. However, Rg1 could prevent these changes or reverse them to some degree. By acting on GR, Rg1 is also a potent stimulator of VEGF expression and this induction is mediated through a PI3K/Akt and β-catenin/T-cell factor-dependent pathway (Leung *et al*., 2006). PI3K/Akt and glycogen synthase kinase 3β are signaling molecules necessary for the Rg1-mediated up-regulation of β-catenin, its translocation into the nucleus, and changes in VEGF expression in HUVECs. Recently Cheung and coworkers (2011) have also shown that in the presence of Rg1, GR and FGFR-1 cooperate to activate a non-genomic signaling cascade that results in angiogenic activity.

Re and Rg2 (Fig. 2), other ginsenosides isolated from different species of Panax, could be also considered as proangiogenic agents. They enhance proliferation, mi-

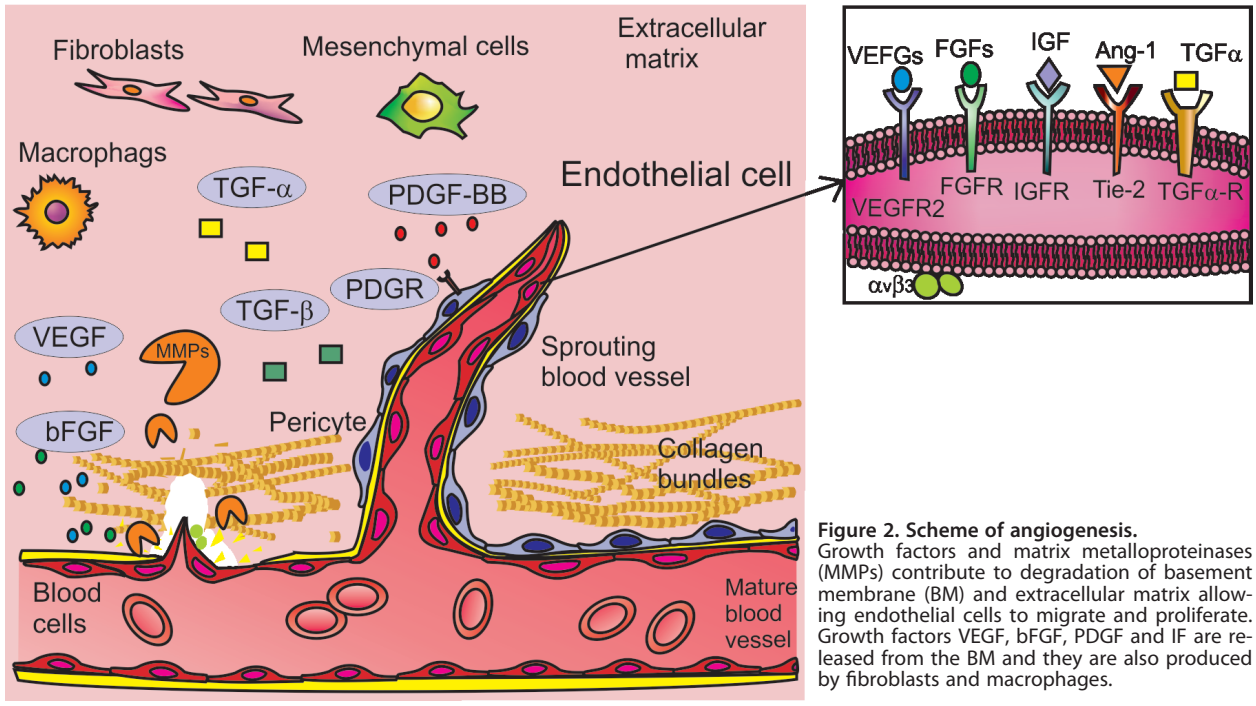

Figure 2. Scheme of angiogenesis.
Growth factors and matrix metalloproteinases (MMPs) contribute to degradation of basement membrane (BM) and extracellular matrix allowing endothelial cells to migrate and proliferate. Growth factors VEGF, bFGF, PDGF and IF are released from the BM and they are also produced by fibroblasts and macrophages.

gration, and tube formation of HUVECs (Huang *et al.*, 2005; Yue *et al.*, 2007; Xin *et al.*, 2006). Additionally, *in vivo* results show that Re incorporated into a genipin-fixed porous acellular bovine pericardium stimulates neovascularization in rats (Huang *et al.*, 2005).

The above data suggests that the natural ginsenosides Rg1, Re and Rg2 isolated from Panax species may be used for the management of wounds.

Aloe vera

Aloe vera is a perennial tropical succulent belonging to the *Liliaceae* family. Although worldwide more than 360 species are known, only a few are currently used by the pharmaceutical and cosmetic industries. Among them, *Aloe barbadensis*, commonly called *Aloe vera*, has been one of the most widely used healing plants in history (Lee *et al.*, 1995). A number of studies have demonstrated positive effects of topical application of *A. vera* on burns and other cutaneous injuries (Davis *et al.*, 1987, 1989, Maenthaisong *et al.*, 2007). Recently, *A. vera* has been proven to accelerate the healing of open wounds in type 2 diabetic radiation-exposed rats when administered orally (Atiba *et al.*, 2010, 2011). Immunohistochemical results have revealed that both TGF-β1- and VEGF-positive cells are increased in *A. vera*-treated animals. *Aloe vera* contains over 100 compounds belonging to glycoproteins, saccharides, anthraquinones, and other low–molecular-weight substances. Among the low–molecular-weight components beta-sitosterol has been identified as an angiogenic factor that may be beneficial to the healing process (Moon *et al.*, 1999). They have demonstrated that crude *A. vera* extract and especially beta-sitosterol stimulate migration of HUVEC cells, show a potent angiogenic activity on the chorioallantoic membrane (CAM) of chick embryos and stimulate neovascularization in the mouse matrigel plug assay (1999). Beta-sitosterol (Fig. 2) from *A. vera* also enhances new vessel formation in gerbil brain damaged by ischaemia/reperfusion, especially in the cingulated cortex and septal regions, in a dose-dependent fashion. In addition, beta-sitosterol enhances the expression of proteins related to angiogenesis, vWF, VEGF, VEGF receptor Flk-1, and blood vessel matrix laminin (Choi *et al.*, 2002). Besides beta-sitosterol, other active components of *A. vera* have been demonstrated to influence angiogenesis. Lee and coworkers (1995) have reported that low–molecular-weight components from a dichloromethane extract of freeze-dried *A. vera* gel stimulate angiogenesis in chick embryo CAM assay. Furthermore, a methanol-soluble fraction (F3) of the gel deserves special attention as it stimulates proliferation and differentiation of artery endothelial cells and increases invasion of calf pulmonary artery endothelial cells into matrigel (Lee *et al.*, 1998). Moreover, the active F3 fraction enhances expression of proteolytic enzymes, especially urokinase-type plasminogen activator and MMP-2, playing a major role in extracellular matrix degradation (Lee *et al.*, 1998). Also acemannan (β-(1,4)-acetylated polymannose) — the major polysaccharide of *A. vera* — stimulates expression of VEGF and other wound healing-related factors (e.g., keratinocyte growth factor-1 and type I collagen) in gingival fibroblasts (Jettanacheawchankit *et al.*, 2009). This can be especially beneficial in the case of oral wound healing. Thus, crude *Aloe vera* extract or isolated proangiogenic components may have potential pharmaceutical applications for the management of wounds.

Hippophae rhamnoides L.

Hippophae rhamnoides L. (family *Elaeagnaceae*) commonly known as seabuckthorn (SBT) is a deciduous shrub native to Europe and Asia (Li & Beveridge, 2003). Leaves, ripe fruits and seeds from seabuckthorn have been found to be a rich source of a large number of bioactive substances including flavonoids (isorhamnetin, quercetin, myricetin, kaempferol and their glycoside derivatives), carotenoids (α, β, δ-carotene, lycopene), vitamins (A, C, E and K), tannins, triterpenes, glycerides of palmitic, stearic and oleic acids and some essential amino acids (Beveridge *et al.*, 1999; Zu *et al.,* 2006). The high content

of bioactive substances has been reflected in its extensive exploitation by traditional medicine. Seabuckthorn has antioxidant (Upadhyay *et al.,* 2010) and anti-inflammatory activity (Ganju *et al.,* 2005) and has been reported to be useful in treating skin wounds (Upadhyay *et al*., 2009a), cardiovascular diseases (Eccleston *et al*., 2002), thrombosis and platelet aggregation (Cheng *et al.,* 2003).

Several studies have also demonstrated an angiogenic activity of *H. rhamnoides* (Gupta *et al.,* 2008; Upadhyay *et al.,* 2010). Upadhyay *et al.* (2010) have indicated that lyophilized aqueous leaf extract of seabackthorn promotes wound healing in experimental burn wounds and has a positive influence on different phases of wound repair, including angiogenesis. That investigation has shown that SBT treatment stimulates angiogenesis in both *in vivo* and *in vitro* models as indicated by histological studies and new vessel formation in chick embryo chorioallantoic membrane model. STB up-regulates VEGF as well as MMP-2 and MMP-9 expression (Ferrara, 1999; Upadhyay *et al.,* 2010). One of the active proangiogenic compounds may be sitosterol which constitutes 57–76 and 61–83%, respectively, of the seed and pulp/peel sterols of the two major subspecies of *H. rhamnoides: sinensis* and *rhamnoides* (Yang *et al*., 2001).

Studies concerning *Hippophae rhamnoides* L.*, Aloe vera* L. and *Curcuma longa* L. have separately shown positive results in wound healing. Gupta *et al.* (2008) investigated the wound healing activity of a poly-herbal formulation (PHF) prepared by combining aqueous lyophilized leaf extracts of *H. rhamnoides* L. and *A. vera* L. and the ethanol rhizome extract of *C. longa* L., in an optimized ratio (1:7:1). Topical PHF treatment brought about an increase of VEGF expression as well as *in vitro* promotion of angiogenesis in the CAM model. However, the promising results showed by Gupta and coworkers need to be further evaluated. It is unknown if the PHF efficacy is due to specific phytoconstituents of one of the plants or is a synergic effect of all components.

Angelica sinensis

Angelica sinensis (*Apiaceae* family) is a key component of the traditional Chinese medicine and, next to ginseng, is one of the most popular herbs. The root of *A. sinensis* is recommended for women for balancing and relief of discomforts such as dysmenorrhea, irregular menstruation, anemia, constipation and abdominal pain (Ososki & Kennelly, 2003). *A. sinensis* contains many bioactive components including ferulic acid (FA), ligustilide, senkyunolide H, senkyunolide I (Lao *et al.,* 2004; Dong *et al.,* 2005), 3-butylphthalide (Li *et al.,* 2006) and polysaccharides (Ye *et al*., 2003). Dong and coworkers (2004) have demonstrated positive effect of *A. sinensis* on inhibition of platelet activation, repair of vascular EC injury, and on improvement of microcirculation in ulcerative colitis. Polysaccharides extracted from *A. sinensis* significantly promote the migration of gastric epithelial cells over an artificial wound (Ye *et al*., 2001a). Ye and coworkers (2001b) suggest that ornithine decarboxylase and c-Myc protein are closely associated with *A. sinensis* improvement of mucosal healing. To date, studies on *A. sinensis* in the context of angiogenesis have shown some discrepancies. Meng *et al.* (2008) have reported that extracts from *Angelica* and *ChuanXiong* could affect VEGF expression in rat myocardial infarction, promote endothelial cell proliferation and increase the number of vessels in chick embryo chorioallantoic membrane models, suggesting that these two herbs have angiogenic activity. Similarly, a study by Lam and colleagues (2008) has indicated that an aqueous extract of *A. sinensis* root enhances HUVEC proliferation, migration, invasion and tube formation on matrigel as well as promotes angiogenesis *in vivo* in zebrafish. This extract promotes angiogenesis through enhancing VEGF expression and stimulating c-Jun N-terminal kinases 1 and 2 (JNK1/2) and p38 phosphorylation to control cell proliferation, viability, and morphogenesis. The above studies are in contrast to a recent investigation by Yeh *et al.* (2011). They have demonstrated that a volatile oil of *A. sinensis* Radix and one of its bioactive components (*n*-butylidenephthalide) have antiangiogenic properties through inhibition of cell cycle progression and induction of apoptosis. However, it has to be mentioned that aqueous extract of *A. sinensis* Radix contains mainly polysaccharides (60%), although ferulic acid, Z-ligustilide and *n*-butylidenephthalide are also detected (Lam *et al*., 2008). On the other hand, volatile oil consists of monoterpenes and sesquiterpenes, and neither ferulic acid nor polysaccharide could be detected (Yeh *et al*., 2011). These findings suggest that distinct constituents present in the different extracts of *Angelica sinensis* Radix are the main reason of the contrasting effects on angiogenesis. This is similar to the opposite activities of Rg1 and Rb1 ginsenosides extracted from various Panax species (Huang *et al*., 2005; Yue *et al*., 2005).

Astragalus membranaceus

Radix Astragali is the dried root of *Astragalus membranaceus* (Fisch) Bunge or *Astragalus membranaceus* var. mongholicus (Bunge) Hsiao (*Fabaceae* family) (Xiao & Liu, 1999). It is one of the 50 fundamental herbs of traditional Chinese medicine (Hoo *et al*., 2010) with a beneficial effect for the strengthening the immune system and for treatment of influenza, abnormal uterine bleeding, diabetes mellitus, and cardiovascular diseases (WHO, 1999). Pharmacological studies have demonstrated cardioprotective effect of *Radix Astragali* against heart failure (Zhao *et al*., 2009), myocardial infarction (Xu *et al*., 2008), chronic hepatitis (Tang *et al*., 2009), and diabetes (Lau *et al*., 2009). Chemical and biological investigations on *Radix Astragali* have identified three major bioactive groups of constituents: isoflavonoids (including formononetin, calycosin, ononin and their glucosides), triterpene saponins (astragalosides I–IV), and polysaccharides (Ma *et al*., 2002).

Radix Astragali has also been shown to have proangiogenic activity. Zhang *et al.* (2009) have demonstrated that *Radix Astragali* extract stimulates HUVEC to proliferate and enhances their motility in the wound healing migration assay via enhancing VEGF mRNA expression and activation of the PI3K-Akt-eNOS pathway. They show that inhibitors of the VEGF-KDR/Flk strongly diminish the effect of *Radix Astragali* extract, suggesting that proangiogenic effects involve the VEGF-KDR/Flk pathway. The compositions of *Radix Astragali* extract includes formononetin, calycosin, (6aR, 11aR)-9,10-dimethoxy-3-hydroxypterocarpan and saponins (astragaloside I, II and IV) comprising 8.15%, 0.77%, 0.01% and 0.88% of the whole extract, respectively. However, calycosin (Fig. 2.) has been found to be the most potent proangiogenic agent among all. Further studies have revealed that calycosin acts as a selective estrogen receptor modulator (Tang *et al*., 2010). It has been shown that phytoestrogens are able to bind to the both isoforms of estrogen receptor (ER) ERα and ERβ (Kostelac *et al*., 2003). Moreover, ERα binds to PI3K, and the stimulation with estrogen leads to activation of the PI3K-Akt-eNOS sig-

naling pathway (Simoncini *et al.*, 2000). Tang and coworkers (2010) have shown that calycosin competitively binds to ERα and ERβ as well as selectively modulates ER transcriptional activities. Activation of ERα and ERβ may affect expression of VEGF and VEGF receptors (Albrecht *et al.*, 2003). Indeed, calycosin has been shown to induce angiogenesis in HUVECs *in vitro* and in zebrafish embryos *in vivo via* the up-regulation of expression of VEGF, Flt-1 and KDR/Flk-1 mRNA. Furthermore, calycosin promotes angiogenesis via activation of MAPK signaling pathways with an involvement of extracellular-signal-regulated kinases 1 and 2 (ERK1/2) (Tang *et al.*, 2010).

Recent data indicate that *Radix Astragali*-derived flavonoids including formononetin, ononin, calycosin, and calycosin-7-*O*-β-D-glucoside increase the level of the inducible subunit of hypoxia-inducible factor mRNA and protein. HIF-1α is a transcription factor that modulates a wide range of processes, including angiogenesis (Zheng *et al.*, 2011). Besides, the above flavonoids reduce the degradation of HIF-1α significantly increasing its level. However, none of the flavonoids induces the phosphorylation of ERK.

The cited studies show promise for further development of *Radix Astragali* as a therapeutic agent for the treatment of wounds and angiogenesis deficiencies. Nevertheless, more research has to be done to accept *Radix Astragali* extract as a regular medicine.

Other examples

Cinnamomum cassia is also one of the medicinal plants that have been used to ease the symptoms of various diseases caused by insufficient vascularization or by insufficient blood circulation. However, only one investigation has been conducted so far on the angiogenic effect of *C. cassia* and its active compound cinnamic acid (Fig. 2). Choi *et al.* (2009) have proven that ethanol extract of *C. cassia* and cinnamic acid induce angiogenesis *in vivo* and *in vitro* by increasing the production of VEGF and up-regulation of Flk-1/KDR receptor expression. Furthermore, the compounds tested increased the vWF antigen expression and hemoglobin contents, which paralleled the onset of angiogenesis and is considered an early indicator of endothelial activation. Similarly to the case of *C. cassia*, despite the beneficial effects in wound healing only single papers have appeared reporting angiogenic activity of extracts from *Stewartia koreana* leaves (Lee *et al.*, 2010), *Uncaria rhynchophylla* (Choi *et al.*, 2005), *Patrinia villosa* (Jeon *et al.*, 2010), *Pueraria montana* (Chung *et al.*, 2010), *Rehmannia glutinosa* (Lau *et al.*, 2009) and *Salvia miltiorrhiza* (Lay *et al.*, 2003a, Lay *et al.*, 2003b; Wu *et al.*, 2010). In all cases significant stimulation of proliferation and migration of HUVECs was observed, which was due to activation of different signaling pathways (Table 1).

DISCUSSION

Currently great efforts are being made to study angiogenesis. In 1994, The Angiogenesis Foundation (http://www.angio.org) declared angiogenesis a 'common denominator' in the most important diseases of the society. This is due to the fact that disruption of angiogenesis leads to pathological states. Thus, excessive angiogenesis contributes to tumor growth, diabetic retinopathy, age-related macular degeneration, psoriasis and endometriosis, and insufficient angiogenesis leads to chronic wounds, coronary artery disease and ischemic heart disease. The intense interest in angiogenesis has resulted in the identification and characterization of at least 20 proangiogenic and more than 30 antiangiogenic factors as well as numerous receptors and signaling partners (Tombran-Tink & Barnstable, 2006). Insufficient angiogenesis is caused by an inadequate production of angiogenesis growth factors and/or excessive amounts of angiogen-

Table 1. Proangiogenic activity of *Panax* sp. extracts and isolated ginsenosides

Species	Common name	Compound	Long-term effects activated	Mechanism of action	References
Panax notoginseng	Sanqi ginseng	Saponin extract of R1, Rg1, Re, Rf, Rg2, b1, Rc, Rb2, b3, Rd, Rg3	Proliferation and tube formation of HUVEC	Enhancing VEGF and KDR/Flk-1 expression; activation of PI3K-Akt-eNOS signaling pathway	Hong *et al.*, 2009
Panax ginseng	Asian ginseng Chinese ginseng	Korean red ginseng water extract of Rb1, Rb2, Rc, Rd, Re, Rf, Rg1, Rg2, Rg3	Proliferation, migration, and tube formation of HUVEC; angiogenesis *in vivo*	Activation of the PI3K/Akt-dependent ERK1/2 and eNOS signaling pathways	Kim *et al.*, 2007
Panax sp.		Pure Rg1	Proliferation, migration and tube formation of HUVEC; endothelial sprouting in the ex vivo rat aorta ring assay; angiogenesis *in vivo*	Decreased expression of ICAM-1, VCAM-1, E-selectin; increased expression of VEGF, RhoA, RhoB, Vav2, IQGAP1, CALM2, LAMA4; PI3K-Akt-eNOS signaling pathway; triggering transcriptional activation of a GRE; GR-dependent activation of FGFR-1	Sengupta *et al.*, 2004 Lü *et al.*, 2004 Yue *et al.*, 2005 Liang *et al.*, 2005 Leung *et al.*, 2006 Lee *et al.*, 1997 Cheung *et al.*, 2011
Panax sp.		Pure Rg2 and Re	Proliferation, migration and tube formation of HUVEC; *in vivo* angiogenesis	PI3K-Akt-eNOS signaling pathway	Huang *et al.*, 2005; Yu *et al.*, 2007, Xin *et al.*, 2006 Furukawa *et al.*, 2006

Table 2. Proangiogenic activity of compounds isolated from different sources

Species	Common name	Family	Compound	Mechanism of action	References
Aloe barbadensis	Aloe vera	Liliaceae	lyophilized powder methanol-soluble fraction (F3) of the gel	Increased expression of TGF-β1, VEGF	Atiba *et al.*, 2010, 20011 Lee *et al.*, 1998
				increased expression of u-PA and MMP-2	Choi S *et al.*, 2002 Jettanacheawchankit *et al.*, 2009
			β-Sitosterol	Increased expression of von Willebrand factor, VEGF, VEGF receptor Flk-1 and laminin	
			acemannan	Increased expression of VEGF	
Hippophae rhamnoides	Seabuckthorn	*Elaeagnaceae*	aqueous leaf extract	Increased VEGF, MMP-2, MMP-9 expression	Upadhyay *et al.*, 2010 Gupta *et al.*, 2008
Angelica sinensis	Dong Quai	*Apiaceae*	aqueous extract containing polysaccharides	Increased VEGF expression; p38 and JNK ½ phosphorylation pathway	Dong et *al.* 2004; Lam *et al.*, 2008; Meng *et al.*, 2008
Cinnamomum cassia	Chinese Cinnamon	Lauraceae	ethanol extract and Cinnamic acid	Increased expression of VEGF and Flk-1/KDR	Choi DY *et al.*, 2009
Astragalus membranaceus		Fabaceae	Radix Astragali extract with calycosin	Increased VEGF expression HIF-1α and , activation of PI3K-Akt-eNOS and VEGF-KDR/Flk pathways	Zhang *et al.*, 2009 Zheng *et al.*, 2011 Tang *et al.*, 2010
			Calycosin	Increased VEGF and KDR/Flk-1 expression; ERK1/2 activation; ; activation of ERα and ERβ and MAPK signaling	
Stewartia koreana		Theaceae	methanol extracts leaves	Stimulation of ERK phosphorylation and Akt kinases	Lee TH *et al.*, 2010
Uncaria rhynchophylla	Cat's Claw	Rubiaceae	ethanol extract of *U. hynchophylla'* s root	Increased VEGF, and bFGF expression and protein secretion of	Choi DY *et al.*, 2005
Salvia miltiorrhiza	Danshen	Lamina-ceae	crude extract and salvianolic acid B	Increased expression of MMP-2, VEGF, VEGF-R2 and Tie-1	Lay *et al.*, 2003a, 2003b
Patrinia villosa	White P.	Valeriana-ceae	aqueous extract	Activation of FAK and Akt signaling pathway	Jeon *et al.*, 2010
Pueraria montana	Kudzu	Fabaceae	aqueous extract	Activation of MEK/ERK-, PI3K/Akt/eNOS-, and Src/FAK-dependent pathways, without altering VEGF expression	Chung *et al.*, 2010

esis inhibitors. Therapeutic angiogenesis, which is aimed at stimulating neovascularization with growth factors, is being developed to reverse these conditions and have been used in clinical trials and animal studies. However, in the field of administration of growth factors in impaired wound healing only one agent has been approved for human use, the recombinant human growth factor BB of platelet origin (rhPDGF-BB) with market name Regranex® (Fonder *et al.*, 2008).

Extensive research has also been carried out in the area of wound healing through the use of medicinal plants since nature is an inexhaustible reservoir of bioactive substances. The traditional folk medicine has highlighted many plants to have a beneficial impact on wound healing and treatment of ischemic heart diseases. Recently, these plants have been analyzed for their impact on the formation of blood vessels *in vitro* and *in vivo*, with special attention given to the influence on VEGF expression. Studies on plants used in traditional medicine have indicated the following species with significant proangiogenic activity: *Aloe vera, Hippophae rhamnoides* L.*, Angelica sinensis, Cinnamomum cassia, Astragalus membranaceus, Stewartia koreana, Uncaria rhynchophylla, Salvia miltiorrhiza, Patrinia villosa* Juss., *Rehmannia glutinosa,* and four ginsengs: *Panax ginseng, P. schinsen*, *P. notoginseng, and P. quinquefolium* (for references see Table 1). Nevertheless, not all of their bioactive components have been isolated and identified. The chemical constituents identified so far belong to polyphenols (cinnamic acid, calycosin, salvianolic acid B), sterols (β-sitosterol), and saponins (ginenosides Rg1, Re, Rg2).

The components of plants described in this review enhance the expression of VEGF and its receptor, VEGFR2 (Moon *et al.*, 1999; Choi S *et al.*, 2002; Lay *et al.*, 2003a; 2003b; Choi DY *et al.*, 2005; 2009; Gupta *et al.,* 2008; Lam *et al.*, 2008; Hong *et al.*, 2009; Zhang *et al.*, 2009; Upadhyay *et al.*, 2010; Tang *et al.*, 2010). They are also able to stimulate angiogenesis through the PI3K-Akt-eNOS pathway. The PI3K-Akt-eNOS signaling has been known as an important determinant of endothelial cell migration, proliferation, and survival (Dimmeler & Zeiher, 1999; Urbich & Dimmeler, 2005). Phosphatidylinositol-3-kinase is an upstream signaling molecule of serine/threonine kinase Akt/protein kinase B. Akt/PKB through phosphorylation of endothelial nitric oxide synthase at Ser1177 stimulates NO production, vasodilation and endothelial cell migration (Somanath *et al.*, 2006).

A second important signaling pathway in angiogenesis is connected with mitogen-activated protein kinases. This pathway is activated by calycosin from *Radix Astragali* (Tang *et al.*, 2010) and extracts from *Angelica sinensis* (Lam *et al.*, 2008), *Stewardia koreana* (Lee *et al.*, 2010), *Panax ginseng* C. A. Meyer (Kim *et al.*, 2007), and *Puerariae montana* (Chung *et al.*, 2010). In the case of the last two plant extracts angiogenesis is stimulated through both MAPK-dependent and PI3K-Akt-eNOS signaling pathways. Mitogen-activated protein kinases are a family of serine/threonine protein kinases which regulate a number of cellular activities (Kuida & Boucher, 2004). Three of the six major subfamilies are especially involved in the growth of blood vessels, and these are extracellular

signal-regulated kinases (ERK1/2), three JUN-amino-terminal kinases (JNK1/2/3) and four p38 protein kinases (p38α/β/γ/δ) (Kuida & Boucher, 2004). ERK1/2 are activated in response to growth factors, whereas JNKs and p38 are usually activated in response to inflammatory cytokines and cellular stress (Robinson & Cobb, 1997). In particular, ERK1/2, one of the major targets of the MAPK signaling pathway, plays an important role in endothelial cell migration and proliferation (Tanaka *et al.*, 1999), additionally the ERKs are activated through the three-component protein kinase cascade Raf→MEK→ERK (Robinson & Cobb, 1997).

One characteristic of crude plant material is that its constituents may have opposite, moderating or enhancing effects. That is why the final activity depends of the interactions among the constituents and the effect of each constituent on its own. The polyherbal formulation is based on this assumption (Gupta *et al.,* 2008). However, it is important to fully characterize and standardize plant extracts because the compositional ratio may have a significant influence on the result such as a pro- or antiangiogenic effect. In contrast to the antiangiogenic effects of ginsenosides such as Rb1 and Rg3, Rg1, Re and Rg2 have been found to be proangiogenic. The latter three increase HUVEC proliferation, migration and tube formation (Sengupta *et al.*, 2004; Huang *et al.*, 2005; Xin *et al.*, 2006; Yu *et al.*, 2007). Similarly, *Angelica sinensis* Radix contains components exerting opposing effects on blood vessel growth (Dong e*t al.*, 2004; Meng *et al.*, 2008; Yeh *et al.*, 2011). Aqueous extract of *A. sinensis* Radix contains mainly polysaccharides (60%) responsible for proangiogenic activity (Lam *et al.*, 2008), and volatile oil consisting of monoterpenes and sesquiterpenes, among them *n*-butylidenephthalide, has been shown to be antiangiogenic (Yeh *et al.*, 2011). These examples emphasize the importance of characterizing active substances present in particular plant extracts for the development of novel angiogenesis modulators as well as the need for standardization of plant-derived medicines, including their methods of preparation. However, elucidation of the molecular signals and pathways that are activated by plant constituents seems to be important as well. The example of resveratrol (*trans*-3,5,4'-trihydroxy stilbene) is particularly interesting. This natural polyphenol primarily extracted from grape and mulberry reveals contrasting effects on angiogenesis that is situation-dependent. In the literature resveratrol is predominantly known as an antitumor agent. However, among the various functions attributed to resveratrol, its influence on angiogenesis is particularly astonishing. Resveratrol has been demonstrated to have anti-angiogenic effects, mainly observed in tumors. It inhibits proliferation of bovine pulmonary artery endothelial cells and HUVECs by inhibition of bFGF- and VEGF-receptor-mediated capillary endothelial cell growth and by increasing p53 protein expression. Also scavenging of reactive oxygen species by resveratrol may contribute to its anti-angiogenic effects. Resveratrol also inhibits formation of new blood vessels *in vivo*, especially during tumor growth (Dulak, 2005; Chen & Tseng, 2007). In addition to its anticancer and anti-angiogenic activity, resveratrol displays a cardioprotective effect. It has been shown that resveratrol protects perfused rat hearts through an increase in inducible nitric oxide synthase (iNOS) (Hattori *et al.*, 2002). Besides, resveratrol enhanced myocardial angiogenesis both *in vivo* and *in vitro* by induction of VEGF, which was regulated by thioredoxin-1 (Trx-1) and heme oxygenase-1 (HO-1). Furthermore, pretreatment of rats with resveratrol (1 mg/kg/day) for 2 weeks reduced infarct size 24 h after myocardial infarction and increased capillary density in the peri-infarct myocardium (Kaga *et al.*, 2005). A strong increase in VEGF, its receptor Flk-1, iNOS, eNOS and redox-regulated transcription factors NF-κB in the resveratrol-treated myocardium was demonstated by Fukuda *et al.* (2006). Resveratrol improves postischemic ventricular function, reduces myocardial infarction and cardiomyocyte apoptosis, activates survival signal, and reduces death signal (Mukherjee *et al.*, 2010). So resveratrol can function both as a pro-angiogenic and anti-angiogenic agent. The dosage and pharmacokinetics of resveratrol but also the cell type are important factors in determining whether it exerts a pro- or antiangiogenic effect (Chen & Tseng, 2007; Mukherjee *et al.*, 2010). At low concentrations resveratrol appears to increase cell proliferation, whereas apoptosis is induced in various cancer cells at higher concentrations. Resveratrol administered at 10–20 mg/kg or 5–10 μM is usually effective to exert protective effects against ischemia-reperfusion injury whereas studies on cancer prevention reveal that resveratrol has to be used at higher concentrations (e.g., 40 mg/kg/day of resveratrol treatment decreases angiogenesis and inhibits tumor growth in gliomas). Taking into account this dose- dependency, resveratrol can be classified as an example of hormetins — mild stress-inducing molecules. The term "hormesis" was introduced by Goldman (1996) to mean "the beneficial effect of a low level exposure to an agent that is harmful at high levels". Another example of a hormetic plant-derived molecule is curcumin (diferuloylmethane) — the active component in the food spice turmeric isolated from the roots of *Curcuma longa*. Administration of curcumin at the dosage of 100 mg/kg body weight resulted in induction of vascularization in rats (Jagetia & Rajanikant, 2003). In mice down-regulation of expression of angiogenic factors was most consistently observed and sustained at 24 h after treatment using the 500 mg/kg dose (Lin *et al.*, 2007). Clinical trials have demonstrated that curcuma extract can be administered safely to patients at doses of up to 2.2 g daily, equivalent to 180 mg of curcumin. However, the main limiting factor is low oral bioavailability in humans and additional intestinal metabolism (Sharma *et al.*, 2001).

The plants reviewed here play an important role in folk medicine. The information about their beneficial effects on wound healing and on the circulatory system comes from the centuries-long tradition. In the development of pharmacological agents there is a need to elucidate the mechanism of their action. The evaluation of angiogenic effects of natural products led to the development of the anticancer drug Taxol®, originally obtained from Pacific yew tree (*Taxus brevifolia*) (Fan *et al.*, 2006). Hence, plant extracts and natural compounds are of substantial interest to researchers as the candidates for novel drugs. The authors cited in this review have shown in *in vitro* and *in vivo* studies the proangiogenic effects of plant extracts and suggested the mechanisms by which they activate the angiogenesis. Despite those promising results more detailed research and thorough standardization of plant extract are necessary to offer novel wound healing therapy. This review indicates the importance of study of the plant effects on angiogenesis not only because of the widely described antiangiogenic effects, but also due to the proangiogenic activity in wound healing and cardiovascular diseases.

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