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ScienceDirect
Journal of Ethnopharmacology 112 (2007) 406–409
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Ethnopharmacological communication

Anti-angiogenic activity of the methanol extract and its fractions of *Ulmus davidiana* var. *japonica*

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Received 23 February 2006; received in revised form 20 February 2007; accepted 2 March 2007
Available online 12 March 2007

Abstract

This study aimed to elucidate anti-angiogenic activity of *Ulmus davidiana* var. *japonica* that has been widely used in folk medicine. The methanol extract (UDE) of *Ulmus davidiana* var. *japonica* concentration-dependently displayed a strong inhibition in the chick chorioallantoic membrane (CAM) angiogenesis. The *n*-butanol fraction of UDE and subsequent 30% MeOH subfraction were identified to be most responsible for the anti-angiogenic activity.

Keywords: *Ulmus davidiana* var. *japonica*; Angiogenesis; Anti-angiogenic; Chorioallantoic membrane; Ulmaceae

1. Introduction

The formation of new blood capillaries from pre-existing capillaries and post-capillary venules, angiogenesis, links with embryonic development and pathological conditions. Diverse unrelated pathological conditions, such as diabetic retinopathy, atherosclerosis, inflammatory diseases, tumor growth and metastasis, are driven by undesirable angiogenesis (Folkman, 1995). Angiogenesis, tightly modulated through a balance of positive and negative regulatory factors, is triggered by pro-angiogenic growth factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and epithelial growth factor (EGF) (Hanahan and Folkman, 1996), which in turn induce activation of their respective receptors on the surface of endothelial cells, resulting in angiogenesis (Risau, 1995).

Since angiogenesis is important in the pathogenesis of various diseases, the inhibition of angiogenesis, or anti-angiogenesis, is one of promising approaches in their treatment. Tumor anti-angiogenesis, playing a prominent role in cancer cell survival, tumor growth and metastasis, is considered to be an important strategy for cancer therapy (Chen et al., 2005). Disruption in the signal pathway to angiogenesis can give rise to the blockage of angiogenesis (Shawver et al., 1997). Since angiostatin (O'Reilly et al., 1994) and endostatin (O'Reilly et al., 1997) were identified to inhibit angiogenesis, there have been a variety of anti-angiogenic components isolated from natural products, such as psammaplin A from a marine sponge (Shim et al., 2004), erianin from *Dendrobium chrysotoxum* (Gong et al., 2004), shiraiachrome A and 11,11′-dideoxyverticillin from *Shiraia bambusicola* (Tong et al., 2004; Chen et al., 2005), epigallocatechin-3-gallate from dried tea leaves (Fassina et al., 2004), pseudolarix acid B from *Pseudolarix kaempferi* (Tan et al., 2004), withaferin A from *Withania somnifera* (Mohan et al., 2004), and geniposide from gardenia (Koo et al., 2004a,b). Some of them are known to inhibit aminopeptidase N, suppress receptor phosphorylation, antagonize VEGF-mediated anti-apoptosis, and disrupt endothelial tube formation.

Ulmus davidiana Planchon var. *japonica* Nakai (Ulmaceae) is a deciduous broad-leaved tree widely distributed in oriental countries, and its stem and root barks have been used as a traditional medicine for the treatment of edema, mastitis, cancer,

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doi:10.1016/j.jep.2007.03.006

inflammation, and rheumatoid arthritis for a long time (Lee, 1966). Until recently, several glycoproteins and terpenoids have been identified in the stem and root barks of *Ulmus davidiana* var. *japonica*, and their pharmacological actions were evaluated. G-120, a 120 kDa glycoprotein isolated from the ethanol extract of the herbal plant, exerted an important role in the induction of apoptosis, suppression of NF-κB activation, and induction of c-Jun/Fra-1or c-Jun/Fra-2 dimerization in MCF-7 human breast caner cells, leading to the inhibited proliferation of the cancer cells (Lee et al., 2005). Although *Ulmus davidiana* var. *japonica* has been used in folk medicine for the treatment of inflammatory diseases and cancer, its anti-angiogenic activity, which is a common treatment target for both disorders, has not been assessed. In the present work, it is demonstrated that *Ulmus davidiana* var. *japonica* possesses potent anti-angiogenic activity.

2. Materials and methods

2.1. Chemicals and fertilized eggs

Retinoic acid and silica gel were purchased from Sigma Chemical Co. (St. Louis, MI, USA). The fertilized eggs used in this work were obtained from Pulmuone Food Co., Seoul, Korea. All other chemicals used were of reagent grade or better.

2.2. Plant material

The stem and root barks of *Ulmus davidiana* var. *japonica* (Ulmaceae) were purchased at Kyungdong Folk Medicine Market, Seoul, Korea, in March 2002, and authenticated by Prof. Ki-Oug Yoo, Division of Life Sciences, Kangwon National University, Chuncheon, Korea. The voucher specimen of the plant material was deposited in the herbarium of the Division of Life Sciences, College of Natural Sciences, Kangwon National University under the acquisition number KWNU56515.

2.3. Preparation of methanol extract and fractions

The barks were ground under liquid nitrogen and extracted for one month with 80% methanol at room temperature. The methanol extract (UDE) was evaporated in vacuo as described in the previous study (Park et al., 2003). The yield was measured to be 41.4%. For solvent fractionation, UDE was suspended in water, and then extracted successively with equal volumes of *n*-hexane (Hex), chloroform (Chf), ethyl acetate (EA) and *n*-butanol (BuOH), leaving residual aqueous fraction (Aq). Each fraction was evaporated in vacuo to yield the residues of Hex (15.2%), Chf (14.1%), EA (12.9%), BuOH (16.6%) and Aq (41.2%) fractions, respectively. The BuOH fraction was further separated by silica gel column chromatography. The subfractions were eluted sequentially with 10% (Fr.1), 20% (Fr.2), 30% (Fr.3), 50% (Fr.4), 70% (Fr.5) and 100% methanol (Fr.6), the yields of which were 13.3, 14.0, 14.8, 17.6, 19.2 and 21.1%, respectively.

2.4. Chorioallantoic membrane (CAM) assay

Anti-angiogenic activity was measured using CAM assay as previously described (Song et al., 2004). The fertilized chicken eggs used in this work were kept in a humidified egg incubator at 37 °C. After 3.5-day incubation, about 2 ml of albumin was aspirated from the eggs through the small hole drilled at the narrow end of the eggs, allowing the small chorioallantoic membrane and yolk sac to drop away from the shell membrane. The shell covering the air sac was punched out and removed by forceps, and the shell membrane on the floor of the air sac was peeled away. In the 4.5-day-old chick embryo, a sample-loaded Thermanox coverslip was applied to the CAM surface. Two days after returning the chick embryo to the incubator, an appropriate volume of 10% fat emulsion (Intralipose, 10%) was injected into a 6.5-day-old embryo chorioallantois. The eggs were then observed under a microscope. The vascular response of each egg was graded as 0, 1+ or 2+. Convergence of a few vessels toward the CAM surface was denoted as 1+, and 2+ reflected an increased density and length of vessels toward the CAM face.

2.5. Statistical analysis

The data were analyzed for statistical significance using Student's *t*-test. *P* values less than 0.05 were considered to be significant.

3. Results and discussion

Angiogenesis contributes to the development and progression of various pathological conditions including tumor growth and metastasis, cardiovascular diseases, inflammatory disease and psoriasis. Angiogenesis can be separated into several main steps, such as degradation of the basement membrane of existing blood vessels, migration, proliferation and rearrangement of endothelial cells, and formation of new blood vessels (Risau, 1995). Down-regulation of angiogenesis has been considered to be advantageous for the prevention of neoplastic growth and inflammation. Some anti-angiogenic substances were identified to be effective in animal models of arthritis, and several antirheumatic drugs, such as indomethacin, methotrexate and corticosteroids, contain anti-angiogenic activity (Tong et al., 2004). Currently, anti-angiogenic strategies are based on inhibition of endothelial cell proliferation, interference with endothelial cell adhesion and migration, and interference with metalloproteinases (Griffioen and Molema, 2000). Many researchers have been trying to screen novel anti-angiogenic principles from various natural products. Up to the present, angiogenesis inhibitors such as fumagillin and minocycline have been isolated from microbial sources (Ingber et al., 1990; Tamargo et al., 1991). Recently, potent anti-angiogenic activities have been identified from *Gardenia jasminoides* fruits (Park et al., 2003), fruiting bodies of the medicinal mushrooms *Phellinus linteus* (Song et al., 2003; Kim et al., 2004) and *Ganoderma lucidum* (Song et al., 2004), and *Cordyceps militaris* (Won and Park, 2005). Genipin, the aglycone of the iridoid glycoside, geniposide, has been convinced to