NF-κB Inhibitory Activities of Phenolic and Lignan Components from the Stems of Acanthopanax divaricatus var. albeofructus

Ya Nan Sun¹, Wei Li², Seok Bean Song³, Xi Tao Yan¹, Seo Young Yang¹, and Young Ho Kim¹,∗

¹College of Pharmacy, Chungnam National University, Daejeon 305-764, Republic of Korea
²School of Biotechnology, Yeungnam University, Gyeongsan, Gyeongbuk 712-749, Republic of Korea
³Gyeongbuk Institute for Bio-industry (GIB), Andong City, Gyeongbuk 760-380, Republic of Korea

Abstract – Acanthopanax divaricatus var. albeofructus (ADA) is commonly ingested as a traditional medicine or as a component of a health drink in Korea. In this phytochemical study, nine phenolics (1 - 9) and three lignans (10 - 12) were isolated from the MeOH extract of the stems of ADA. Chemical structures were elucidated by comparing spectroscopic data with reported values. Nuclear factor kappa B (NF-κB) inhibitory activity of the isolated compounds was evaluated using an NF-κB luciferase assay in HepG2 cells. Among them, compounds 1, 3 - 8, and 11 showed significant inhibitory effects on TNFα-induced NF-κB transcriptional activity in a dose-dependent manner, with IC₅₀ values ranging from 13.25 to 37.36 µM. Further studies on potential anti-inflammatory effects and the benefits of phenolic and lignan components from ADA are warranted.

Keywords – Acanthopanax divaricatus var. albeofructus (ADA), Araliaceae, NF-kappa B, phenolic, lignin

Introduction

Acanthopanax divaricatus var. albeofructus (ADA) is a deciduous shrub of the Araliaceae family that has been used in traditional oriental medicine due to its tonic and prophylactic functions. In Korea, the leaves, stems and roots of this species have been ingested as a drink and drug. ADA has demonstrated both anticancer and antivirus effects, as well as other functions. In addition, during the course of previous studies, the water extract of ADA fruits has been applied to human peripheral blood mononuclear cells (hPBMC). Moreover, both water and ethanol extracts of ADA leaves have demonstrated antioxidative activity in human dermal fibroblasts.¹-⁴ As well as Lyu et al. further investigated the antioxidant activity of various components of ADA fruits.⁵ Phytochemical studies on ADA have determined that its main active constituents are triterpenoids, triterpenoidal saponins, lignans and cyanidins. However, no active NF-κB inhibitory components have not been reported to date. With regards to the Acanthopanax genus, recent reports have shown that impressic acid from the leaves and some phenolics from the stems of A. koreanum display significant NF-κB inhibitory activity.⁶,⁷ Therefore, this study is the first to report NF-κB transcriptional inhibitory activities of the phenolic and lignan components of ADA, an important finding for both medicine and food science research.

Nuclear factor kappa B is a protein complex that controls DNA transcription. It is found in nearly all animal cell types and is involved in cellular responses to stimuli, such as stress, cytokines, free radicals, ultraviolet irradiation, and bacterial or viral antigens.⁸-¹¹ Over the past few years, the transcription of NF-κB, and its regulatory proteins, has emerged as a signaling system of preeminent importance in human physiology and in an increasing number of pathologies. NF-κB plays a role in many compartments of the immune system during immune cells differentiation, immune activation, and in the development of lymphoid organs.¹² Furthermore, activation of NF-κB causes transcription at the κB site, which is involved in several diseases, including inflammatory disorders and cancer. Hence, NF-κB signaling inhibition is an important therapeutic target for the treatment of such diseases.¹³ In the present study, twelve compounds were isolated from ADA. The effects of compounds 1 - 12 on TNF-α induced NF-κB transcriptional activity were evaluated in human hepatocarcinoma (HepG2) cells were evaluated using an NF-κB luciferase assay.

*Author for correspondence
Young Ho Kim Ph.D., College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea
Tel: +82-42-821-5933; E-mail: yhk@cnu.ac.kr
Experimental

General experimental procedures – The NMR spectra were recorded using a JEOL ECA 600 spectrometer (1H, 600 MHz; 13C, 150 MHz), with tetramethylsilane (TMS) as an internal standard. Heteronuclear multiple quantum correlation (HMQC), heteronuclear multiple bond correlation (HMBC), and 1H-1H correlation spectroscopy (COSY) spectra were recorded using a pulsed field gradient. EI-MS spectra were obtained using A Hewlett Packard HP 5985B spectrometer, ESI-MS using an Agilent 1200 LC-MSD Trap spectrometer. Melting points were determined using an Electro thermal IA-9200 system. Column chromatography was performed using a silica gel (Kieselgel 60, 70 - 230, and 230 - 400 mesh, Merck, Darmstadt, Germany), YMC RP-18 resins, and thin layer chromatography (TLC) was performed using pre-coated silica-gel 60 F254 plates (both 0.25 mm, Merck, Darmstadt, Germany), the spots were detected under UV light and using 10% H2SO4.

Plant material – The stems of Acanthopanax divaricatus var. albofructus (ADA) were collected from the herbal garden, College of Pharmacy, Chungnam National University in Daejeon, Korea in September 2012 and were identified by Prof. Young Ho Kim, Chungnam National University. A voucher specimen (CNU12104) was deposited at herbarium, College of Pharmacy, Chungnam National University, Korea.

Extraction and isolation – Dried stems of ADA (6.0 kg) were extracted three times with MeOH under reflux. The MeOH extract (300.0 g) was suspended in H2O (1.6 L) and partitioned with n-hexane and EtOAc (each 1.6 L × 3). The EtOAc extract (40.0 g) was subjected to silica gel column chromatography with a gradient of CHCl3-acetone-MeOH (1:0:0 to 1:1:0.2), to yield seven fractions (Fr. A1-A7). Sub-fraction A2 was further purified by reverse-phase (RP) column chromatography with a MeOH-H2O (1:2.5 to 1:1) elution solvent to yield compounds 1 (15.0 mg) and 8 (5.0 mg). Fr. A3 was further chromatographed in a silica gel column, using a gradient of n-hexane-EtOAc-MeOH (6:1:0.1 to 1:1:0.2), to yield eight sub-fractions (Fr. A3.1-A3.8). Further purification of Fr. A3.3 was conducted by chromatography in an RP column with an eluent gradient of MeOH-H2O (0.8 : 1 to 2.2 : 1) to yield compounds 2 (6.0 mg), 3 (40.0 mg), and 5 (49.0 mg). Fr. A3.5 was further chromatographed in an RP column with MeOH-H2O (0.9 : 1) to yield compound 6 (2.0 mg). Fr. A5 was chromatographed in a silica gel column with an eluent gradient of n-hexane-EtOAc-MeOH (2.3 : 1 : 0.1 to 1 : 1 : 0.1) to yield four fractions (Fr. 5.1-5.4). Fr. A5.2 was column chromatographed in an RP column, eluting with MeOH-H2O (1 : 1) to yield compound 4 (20.0 mg). The H2O fraction (100.0 g) was chromatographed on a column of highly porous polymer (Diaion HP-20), and eluted with H2O and MeOH (1 : 0.5 : 1 : 1 : 1 : 3 : 0 : 1) to yield five fractions (Fr. B1-B5). Fr. B2 was subjected to silica gel column chromatography with a gradient of CHCl3-MeOH (10 : 1 to 0 : 1) to yield six fractions (Fr. B2.1-B2.6). Fr.B2.5 was separated using an RP column with a MeOH-H2O (1 : 1) elution solvent to yield compound 9 (5.0 mg).

Fr. B3 was separated by silica gel column chromatography with a gradient of CHCl3-MeOH-H2O (6.5 : 1 : 0.1 to 0 : 1 : 0) to yield four sub-fractions (Fr. B3.1-B3.4). Fraction B3.2 was further chromatographed by silica gel chromatography column with EtOAc-MeOH (10 : 1 to 1 : 1) to yield compounds 7 (7.0 mg), 10 (52.0 mg), and 11 (10.0 mg). Fraction B3.4 was further chromatographed on an RP chromatography column with MeOH-H2O (1.5 : 1) to yield compound 12 (10.0 mg).

Cell culture and reagents – Human hepatocarcinoma HepG2 cells were maintained in Dulbecco's modified Eagles' medium (DMEM) (Invitrogen, Carlsbad, CA) containing 10% heat-inactivated fetal bovine serum (FBS), 100 units/mL penicillin, and 10 μg/mL streptomycin at 37 °C and 5% CO2. Human TNF-α was purchased from ATgen (Seoul, Korea).

Cytotoxicity Assay – A Cell-Counting Kit (CCK)-8 (Dojindo, Kumamoto, Japan) was used to analyze the effect of compounds on cell toxicity according to the manufacturer’s instructions. HepG2 cells were cultured overnight in a 96-well plate (~1 × 104 cells/well). Cell toxicity was assessed after the addition of compounds in a dose-dependent manner. After 24 h of treatment, 10 μL of the CCK-8 solution was added to triplicate wells and incubated for 1 h. The absorbance at 450 nm was measured to determine the viable cell numbers.

NF-κB-luciferase Assay – The luciferase vector was first transfected into human hepatocarcinoma HepG2 cells. After a limited amount of time, the cells were lysed, and luciferin, the substrate of luciferase, was introduced into the cellular extract along with Mg2+ and an excess of ATP. Under these conditions, luciferase enzymes expressed by the reporter vector could catalyze the oxidative carboxylation of luciferin. Cells were seeded at 2 × 105 cells per well in 12-well plates and grown. After 24 h, cells were transfected with inducible NF-κB luciferase reporter and constitutively expressing Renilla reporter. After 24 h of transfection, medium was changed to assay medium (Opti-MEM + 0.5% FBS + 0.1 mM NEAA + 1