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Antioxidant and free radical scavenging activities of taxoquinone, a diterpenoid isolated from *Metasequoia glyptostroboides*

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ABSTRACT

Nowadays, there is an upsurge of interest on finding effective phyto-constituents as new sources of natural antioxidants for using in food and pharmaceutical preparations. However, this study was carried out to investigate the antioxidant and free radical scavenging efficacy of a biologically active diterpenoid compound taxoquinone isolated from *Metasequoia glyptostroboides* in various antioxidant models. An abietane type diterpenoid taxoquinone, isolated from ethyl acetate cone extract of *Metasequoia glyptostroboides*, was analyzed for its antioxidant efficacy as reducing power ability and its ability to scavenge free radicals such as 1,1-diphenyl-2-picryl hydrazyl (DPPH), nitric oxide, superoxide and hydroxyl radicals. As a result, taxoquinone showed significant and concentration-dependent antioxidant and free radical scavenging activities of DPPH, nitric oxide, superoxide and hydroxyl free radicals by 78.83%, 72.42%, 72.99% and 85.04%, as compared to standard compounds ascorbic acid (81.69%, 74.62%, 73% and 73.79%) and α-tocopherol/butylated hydroxyanisole (84.09%, 78.61%, 74.45% and 70.02%), respectively. These findings justify the biological and traditional uses of *M. glyptostroboides* or its secondary metabolite taxoquinone as confirmed by its promising antioxidant and free radical scavenging activities.

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1. Introduction

Overproduction of free radicals and reactive oxygen species (ROS) has been confirmed in a human body due to the perturbation of various metabolic reactions (Hancock et al., 2001). Free radicals and ROS are generated through normal reactions within the body during respiration in aerobic organisms which can exert diverse functions like signaling roles and provide defense against infections (Hancock et al., 2001). However, many degenerative human diseases including cancer, cardio- and cerebrovascular diseases have been recognized being a possible consequence of free radical damage to lipids, proteins and nucleic acids (Choi and Lee, 2009). Natural antioxidants protect the living system from oxidative stress and other chronic diseases, therefore they can play an important role in health care system (Lopez et al., 2007).

The food industry has long been concerned with issues such as rancidity and oxidative spoilage of foodstuffs (Shahidi and Wanasundara, 1992). The auto-oxidation of lipids during storage and processing resulting in the formation of various free radicals, is the major reaction responsible for the deterioration in food quality affecting the color, flavor, texture and nutritive value of the foods (Choi and Lee, 2009). Hence, antioxidants are often added to foods to prevent the radical chain reactions of oxidation by inhibiting the initiation and propagation steps leading to the termination of the reaction and a delay in the oxidation process (Thorat et al., 2013).

Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butylhydroxyquinone (TBHQ) effectively inhibit the formation of free radicals and lipid oxidation. However, these frequently used synthetic antioxidants are restricted by legislative rules due to their being toxic and carcinogenic by nature (Shahidi and Wanasundara, 1992). Therefore, there has been a considerable interest in food practices and a growing trend in consumer preferences for using natural antioxidants over synthetic ones in order to eliminate synthetic antioxidants in food applications (Thorat et al., 2013), giving more emphasis to explore natural sources of antioxidants. This has led to develop a huge working interest on natural antioxidants by both food scientists and health professionals. Nowadays, there has been a convergence of interest among researchers to find out the role of natural antioxidants in the diet and their impact on human health (Formanek et al., 2001).

Metasequoia glyptostroboides Miki ex Hu is a deciduous coniferous tree of the redwood family, Cupressaceae. This species of the genus *Metasequoia* has been propagated and distributed in many parts of

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Eastern Asia and North America, as well as in Europe. Previously we reported various biological properties of various essential oils derived from *M. glyptostroboides* such as antibacterial (Bajpai et al., 2007a), antioxidant/antibacterial (Bajpai et al., 2009a), antidermatophytic (Bajpai et al., 2009b) and antifungal (Bajpai et al., 2007b) activities. In addition, the antibacterial activities of terpenoid compounds isolated from *M. glyptostroboides* have also been reported against foodborne pathogenic bacteria (Bajpai et al., 2010; Bajpai and Kang, 2011a, 2011b).

The biological efficacy of *M. glyptostroboides* has been reported previously *in vitro* and *in vivo* both, however, no research has been reported on the antioxidant and free radical scavenging efficacy of taxoquinone from *M. glyptostroboides*. Hence, in our continuous efforts to investigate the efficacy of biologically active secondary metabolites, in this study, we assayed the antioxidant, and free radical scavenging efficacy of taxoquinone, an abietane type diterpenoid isolated from *M. glyptostroboides*.

2. Materials and methods

2.1. Chemicals and instruments

The chemicals and reagents used in this study such as 1,1-diphenyl-2-picryl hydrazyl (DPPH), sodium nitroprusside (SNP), Griess reagent, trichloroacetic acid (TCA), nitro blue tetrazolium (NBT), ferric chloride, potassium ferricyanide and gallic acid as well as standard antioxidant compounds ascorbic acid, butylated hydroxyl anisole (BHA) and α-tocopherol were purchased from Sigma-Aldrich (St. Louis, USA) and were of analytical grade. Spectrophotometric measurements were done using a 96-well microplate reader (Infinite M200, Teacan, Mannedorf, Switzerland).

2.2. Plant material

The cones of *M. glyptostroboides* were collected locally from Pohang city, Republic of Korea, in November and December 2008, and identified by the morphological features and the database present in the library at the Department of Biotechnology, Daegu University, Korea. A voucher specimen (DUB-0038) was deposited in the herbarium of the College of Engineering, Department of Biotechnology, Daegu University, Korea.

2.3. Extraction, isolation and purification of taxoquinone

Dried cones of *M. glyptostroboides* (2 kg) were milled into powder and then extracted with ethyl acetate at room temperature for 12 days. The extract was evaporated under reduced pressure using a rotary evaporator (EYELA N1000, Japan). The dried ethyl acetate extract (7 g) was subjected to column chromatography over silica gel (mesh 230–400 mesh, Merck, Darmstadt, Germany) and was eluted with hexane-ethyl acetate-methanol solvent system to give 20 fractions. Of the fractions obtained, fraction-12 was further purified by preparative TLC over silica gel GF_{254} using hexane-ethyl acetate (1:2) as a mobile phase to give one compound (152 mg) which on the basis of spectral data analysis was characterized as taxoquinone as shown in Fig. 1. (Bajpai et al., 2010).

2.4. Determination of DPPH radical scavenging efficacy

The antioxidant activity of taxoquinone, based on the scavenging of stable DPPH free radical, was determined by the method described previously with a minor modification (Braca et al., 2001). Different concentrations of taxoquinone and reference compounds such as ascorbic acid and α-tocopherol (25–150 μg/mL) were added to 0.004% methanolic solution of DPPH (1:1) in a 96-well microplate. The mixture was incubated at 37 °C in dark for 30 min with shaking at 150 rpm. Absorbance was recorded at 517 nm using the 96-well microplate reader against a blank sample containing only methanol. All the tests were run in triplicate. Ascorbic acid and α-tocopherol were used as reference compounds. The percent inhibition activity was calculated using the formula:

$$\text{Inhibition } (\%) = [(\text{A control} - \text{A test})/(\text{A control})] \times 100,$$

where, A control is the absorbance of the control reaction at 517 nm and A test represents the absorbance of a test reaction at 517 nm.

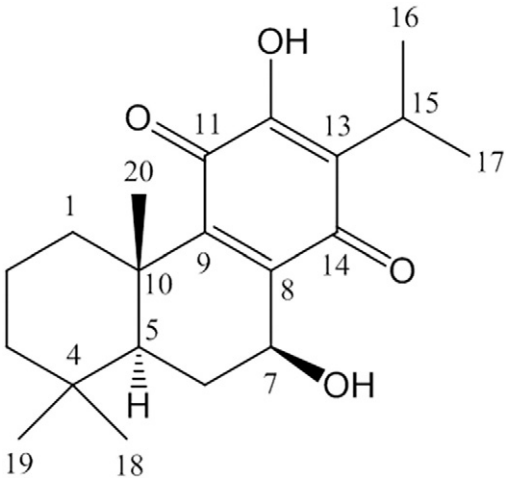

Fig. 1. Chemical structure of taxoquinone, a diterpenoid isolated from *Metasequoia glyptostroboides*.

2.5. Determination of nitric oxide (NO) radical scavenging efficacy

In aqueous solution at physiological pH, SNP automatically generates NO, which intermingles with oxygen to generate nitrite ions that can be anticipated by the Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) (Green et al., 1982). Scavengers of free radicals result in the reduced production of NO. In this assay, the solution of SNP (10 mM) in phosphate buffer saline (PBS pH 7.4) was mixed with different concentrations of taxoquinone, ascorbic acid and α-tocopherol (20–100 μg/mL). The mixture was incubated at 37 °C for 60 min in light. The half quantity of aliquots was taken and mixed with equal quantity of the Griess reagent, and the mixture was incubated at 25 °C for 30 min in dark. The absorbance of pink chromophore generated during diazotization of nitric ions with sulphanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride (NED) was read at 546 nm against a blank using only methanol (Green et al., 1982). All the tests were performed in triplicate. Ascorbic acid and α-tocopherol were used as standard reference compounds. The percent inhibition activity was calculated using the formula:

$$\text{Inhibition } (\%) = (\text{A control} - \text{A test})/(\text{A control}) \times 100,$$

where, A control is the absorbance of the control reaction at 546 nm and A test represents the absorbance of a test reaction at 546 nm.

2.6. Determination of superoxide radical scavenging assay

Superoxide radical scavenging activity of taxoquinone was measured by the reduction of nitro blue tetrazolium (NBT) according to the previously reported method with minor modifications (Fontana et al., 2001). In this assay, the non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which reduce NBT to a purple color formazan. The reaction mixture (150 μL) contained phosphate buffer (0.2 M, pH 7.4), NADH (73 μM), NBT (50 μM), PMS (15 μM) and various concentrations

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