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Antioxidant Activity and Anti-inflammation Action of Orchid leaf ethanol Extracts

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Abstract

Objectives*: Orchid has excellent efficacy in the treatment of antioxidants and is widely used in folk remedies Therefore, we want to observe the possibility of implantation as an additive to cosmetics.*

Methods*: Cell toxicity tests were performed to observe the safety of the orchid leaf ethanol extracts, and DPPH solution was used to observe the antioxidant power of the components themselves. The effect of fluorescence on the expression of DCF-DA was observed. The anti-inflammatory efficacy was observed by observing the potential as an inhibitor of NO produced by inflammatory reactions.*

Findings*: Orchid leaf ethanol extracts was considered to be a safe substance because it did not show cytotoxicity, and DPPH was used to suppress oxidative stress inhibition in a concentration-dependent manner. Inhibition of ROS production in RAW 264.7 cells expressed in the fluorescent substance DCF-DA showed excellent antioxidant activity of the ethanol extract of orchid leaves. NO production was inhibited in LPS-induced RAW 264.7 cells but weakly at low concentrations. However, as the concentration increased, NO production was inhibited, which may be anti-inflammatory effect.*

Applications*: These results suggest that orchid leaf ethanol extracts is not cytotoxic, and it is highly to be used as an anti-aging cosmetic ingredient due to its excellent antioxidant and anti-inflammatory effects.*

Keywords: *Orchid, cell-toxicity, DPPH, ROS, NO, Cosmetic*

1. INTRODUCTION

Recently, there is a growing interest in nature-derived drugs obtained from nature. Nature is the most invaluable source of medicinal compounds as huge chemical diversity is present in millions of species of plants, marine organisms, animals, and micro-organisms[1]. The Orchids grow all over the world except in the polar regions, especially It grows well in the tropical cloud forests. Beautiful, fragrant flowers bloom, various species of an ornamental plant, which is one of the most evolved plants of a single-seed leaf plant, and is a perennial plant. About 25,000 species are known around the world, and Korean native species are 84 species. In addition, orchids

are well known for their medicinal use, and a total of 365 kinds of plants, including some orchids, are listed in the Chinese Materia Medicine[2]. The northeastern part of India is a hot spot famous for about 876 species of orchids in 151 and accounts for about 70 percent of India's total orchids. People in the region use orchids in various folk medicines to treat them as they are found to be rich in flavonoids glycosides, carbohydrates and other phytochemicals[3]. Extracts and metabolites isolated from orchids have been found to have useful therapeutic activities such as diuretic, antibacterial, anti-inflammatory, anti-inflammatory, hypo-inflammatory, anti-microbial, neuroprotective[4]. The Orchidaceae includes five subfamilies(Apostasioideae Horaninov, Cypripedioideae Kosteletzky, Epidendroideae Kosteletzky, Orchidoideae Eaton, Vanilloideae Szlachetko) and many tribes and subtribes[2]. Recently, it is known as an effective ingredient for skin whitening. [5]. In addition, orchid is known to cause various diseases such as inflammation, skin aging, cancer, and inhibits the oxidative stress action that damages tissues by destroying DNA, substrate, and enzymes in the body[6].

Oxidative stress can be increased by metabolic stress, tissue damage, and a continuous cycle of apoptosis, leading to increased reactive oxygen production, further exacerbating oxidative stress[7]. However, free radicals are not necessarily harmful to the human body. There are also three types of enzymes (SODs) that convert ions of excess oxidation into oxygen and hydrogen peroxide. Their enzymes contain metal ions such as copper, manganese, and zinc. Currently, BHA (butylated hydroxy anisole), PG (progylgallate), BHT(butylated hydroxy toluene) and TBHQ (t-butylhydroquinone) have been used for synthetic antioxidants, but there is a growing interest in natural antioxidants as safety issues arise, such as avoidance of compounds and toxic effects in heavy use[8]. The skin originally has a very good antioxidant defense. However, it is not possible to protect damaged tissues from free radicals. Therefore, it is recommended to slow down aging by minimizing oxidative stress through antioxidants[6].

Therefore, the possibility of natural antioxidants and anti-inflammatory agents using Orchids leaf ethanol extracts belonging to the polyphenolic compound is examined from the point of view of spices to determine whether it can be a cosmetic material.

2. MATERIALS AND METHODS

2.1 Reagent and Cell culture

3-(4,5-dimethyliazol-2-yl)-2, 5-diphenyl tetrazolium bromide(MTT), 2',7'-dichlorofluorescin diacetate(DCF-DA) was purchased from the Molecular Probe Co. (Eugene, OR, USA). Raw 264.7 was purchased from Seoul National University's Cell Bank. Raw 264.7 macrophage was grown at a concentration of 37°C with 10 percent phthalate serum and a 5 percent concentration of phenicillin/streptomysin (100IU/ 50μg/mL).

2.2 Experiment material

Orchids, which were used as test materials for this study, were grown directly, 500g of starch was removed, roots, stems and flowers were removed, and cleaned, dried, then 4 times of 95 percent ethanol solution was added to the samples, and then filtered at room temperature. A week later, was filtered twice with Wattman filter paper and then with sterile filter paper. Remove ethanol using rotabapo (50-55 rpm) and obtain 2.5 g of extract to use as a sample.

2.3 Cell toxicity measurement using MTT.

To confirm the cell toxicity of orchid leaf ethanol extracts, the MTT method was applied. Raw 264.7 macrophage was used, and divided 1 X 10^4cells per well in 96 well plates, cultivated for 24 hours, added sample by concentration, and cultivated at 37℃, CO_2incubator for 72 hours. After 72 hours, the cell culture solution was removed, and 1 mL of 500μg/mL of MTT solution dissolved in Krebs buffer solution (mM :NaCl 137, KCl 2.7, $Na2HPO_4$0.4, $MgCl_2$0.5, HEPES [pH 7.4] 10, $CaCl_2$1.8, glucose 5) to each well and cultivated for 4 hours in dark. Then, the supernatant was removed, and 200 μL of DMSO was added to each well to dissolve MTT formazan. After completely dissolving MTT formazan for 15 minutes in room temperature, the absorbance was measured in 570nm.

2.4 DPPH radical scavenging activity

180μL of 0.1mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution dissolved in ethanol to 96 well plates orchid leaf extracts prepared in each concentration was added 20μL each, cultivated for 30 minutes in 37℃ in the dark were processed to absorbance measurement in 517 nm using FL 600 ELISA microplate reader (BioTek, Winooski, VT, USA).

DPPH radical scavenging activity(%) = 100 - {(Absorbance of added/Absorbance of non-added) ×100}

2.5 Intracellular oxidation stress measurement

The measurement applied principle of DCF-DA joining the RAW 264.7 cell to react with free radical generated in cell to oxidize into DCF, the fluorescent material. After suspending RAW 264.7 cell to 15mL of Krebs buffer solution, 20 μM of DCF-DA was added and cultivated for 1 hour in dark. After cleaning with Krebs buffer solution without DCF-DA, cell was extracted by centrifuging. It was divided into 1 X 10^5 cells/mL, preprocessed by concentration, and added silica 1 ㎎/mL to induce H_2O_2 production for 30 minutes. After centrifugation, the cell pellet was redivided to 200 μL Krebs buffer solution, transported to 96 well plates and measured its fluorescence (Ex 485 nm/ Em 535 nm).

2.6 Intracellular nitric oxide measurement

RAW 264.7 cell was divided 1ml to each well as 1 X 10^5 cells/mL to 24 well plates. After mixing 100μL of cell culture supernatant and 150μ of Griess reagent(1% sulfanilamide in 5% phosphoric acid + 1% α-naphthylamide in H_2O) to 96 well plates, and reacted 5 minutes and used ELISA microplate reader(MQX200R, BioTek, Winooski, VT, USA) to measure absorbance in 540nm. To create calibration curve, the study used sodium nitrite($NaNO_2$) as standard for comparison.

2.7 Data analysis and statistical verification

This experiment statistical analysis was performed using SPSS Window Version 17.0 (SPSS Inc., Illinois, USA), and the significance was tested by Student's t-test. Was carried out three times or more independently under the same conditions noted in Mean ± standard deviation (Mean ± SD), the experiment determined that there was a statistically significant difference when the p value was less than 0.05.

3. Results and Discussion

3.1 Cell toxicity assay

To observe cell toxicity of the orchid leaf ethanol extracts, samples were prepared at a concentration of 25, 50, 100μg/ml and treated with Raw 264.7 cells to measure cell toxicity. The cell toxicity of orchid leaf extracts by MTT assay was 97% at 100μg/mL[Figure 1]. These results are similar to those of a study in which *D. formosum* ethanol extractor reduced cytotoxicity in a concentration-depentent manner[9]. In another study, ochid ethanol extracts clearly showed that data obtained from MTT analysis could potentially induce cell toxicity to DL cells without *D. formosum* ethanolic extract affecting normal cells. Denbinobin, obtained from *ephmerantha lonchophylla*, was found to reduce the cell viability of the human colorectal cancer method, as measured by MTT analysis[10]. Thus, the orchid leaf ethanol extracts used in this study did not show cell toxicity at all concentrations, so it was possible to verify stability as a cosmetic material.

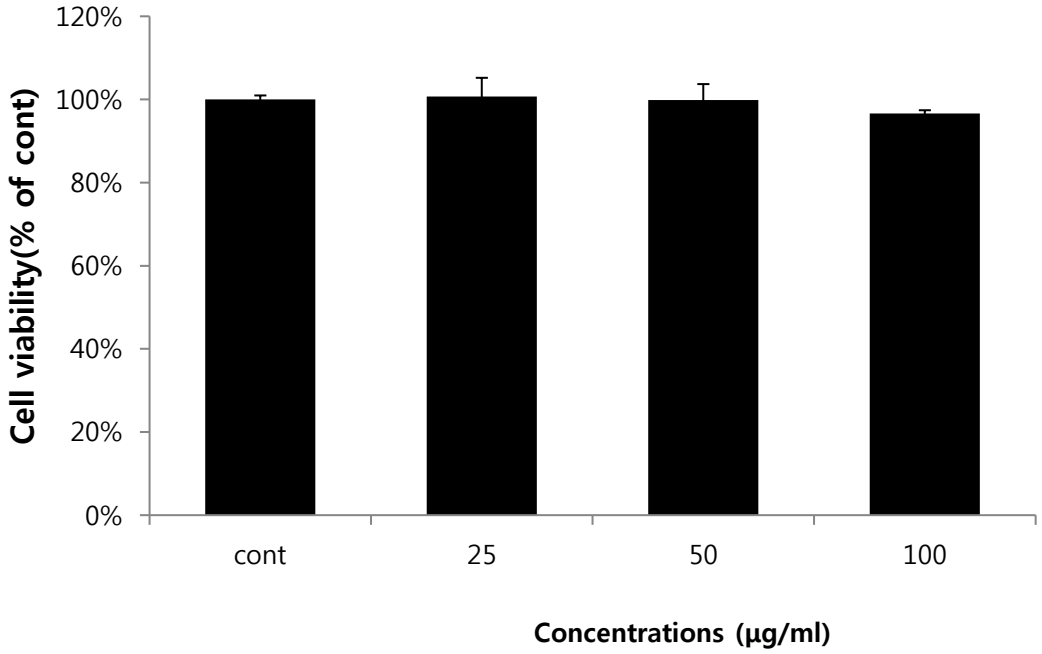

Figure 1. Cell toxicity of Orchid leaf ethanol extracts in Raw 264.7 macrophage. Results are means ± SD from four separate experiments.

3.2 Free radical scavenging activity

Oxidation stress can be amplified by a series of cycles of metabolic stress, tissue damage and cell apoptosis, increasing free-radical production and damaging free-radical suppression and scavenger low systems, further aggravating oxidation stress[7]. Among the various components that reduce oxidative stress, flavonoid glycoside, and others are known as typical antioxidants, and orchids also contain these ingredients. Orchid leaf ethanol extracts is known to have antioxidant properties and was DPPH assay to observe antioxidants and activity on its own. At 25μg/mL concentrations, the results were mild with 5% oxidative stress control, but at 50μg/mL and 100 μg/mL, the results were 26% and 43% oxidative stress control, respectively. These results can be inferred from the antioxidants contained in orchids, and the extract itself is believed to be fully available as an antioxidant. [Figure 2].

In the previous study, the antioxidant power of orchid leaf ethanol extract itself was measured, and DCF-DA fluorophore was used to inhibit ROS formation in RAW 264.7 macrophages derived from 1 mg/mL of silica as a stimulant to measure the antioxidant activity in cells. The results showed that orchid leaf ethanol extracts had a very strong inhibitory effect on the production of ROS in cells at all concentrations. The cellular oxidation inhibitors inhibited ROS generation at low concentrations of 25μg/mL and inhibited generation of 24% and 29% respectively at concentrations of 50μg/mL and 100μg/mL[Figure 3]. These results are believed to be the action of various antioxidant components containing orchid leaf ethanol extracts, which are known to be excellent materials not only for the antioxidant power of the extracts itself but also for the ability to inhibit ROS production within the cell, and can be used as antioxidant and antiaging components in cosmetics.

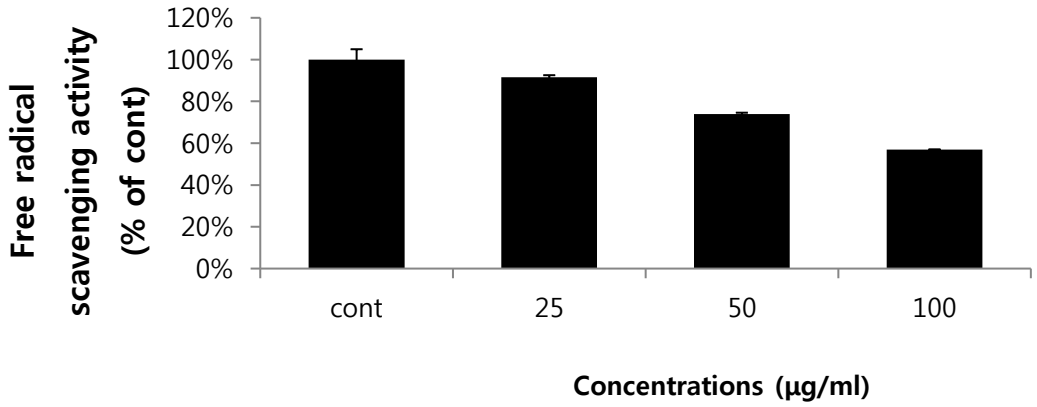

Figure 2. Antioxidant activity of Orchid leaf ethanol extracts in DPPH Assay. Results are means ± SD from four separate experiments.

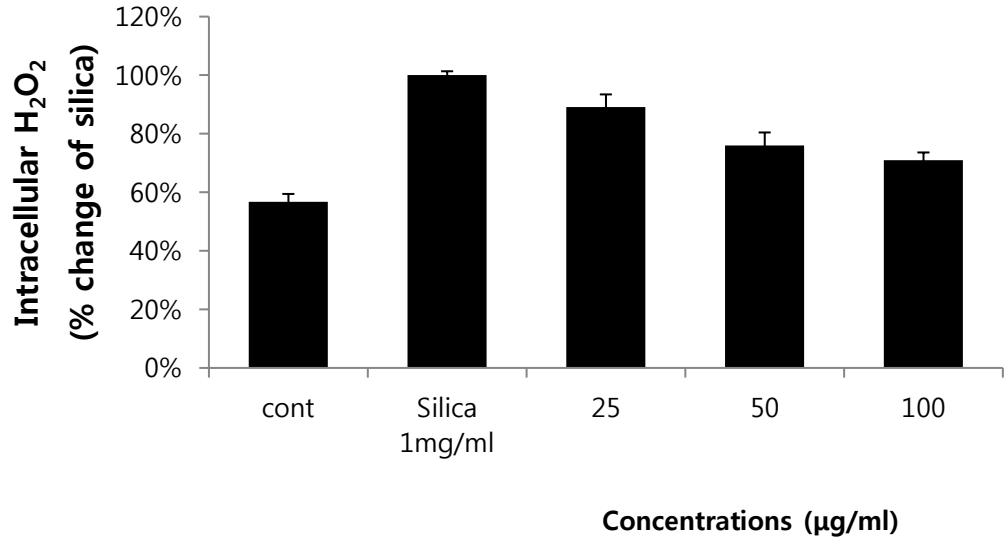

Figure 3. Antioxidant activity of Orchid leaf ethanol extracts 1 mg/mL silica-induced ROS production in Raw 264.7 macrophage. Results are means ± SD from four separate experiments.

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3.3 Nitric oxide product inhibition activity

Inflammation is a complex biological response to bodily tissue damage by a pathogen, damaged cell, or stimulant. Inflammation is a defensive reaction that includes immune cells, blood vessels, and molecular intermediates. The function of inflammation is to remove the cause of cell damage and damaged tissue and necrotic cells and to help repair the tissue[11]. Reactive oxygen species are representative as inflammatory mediators that cause inflammation, and other nitric oxides, prosthetic acids (PGs), cytokines and the like are known. Oxidation produces prosthelandin E2, nitric oxide, tumor necrosis factor-a, and interleukin-6, nitrite, which is synthesized by nitric oxide in tissues and cells and regulates immune function, vasodilation, neurotransmission, and blood coagulation. It is known to have a function[12]. Nitric Oxide is a colorless gas that is oxidized to nitrogen and NO is one of the active oxygen and is a very unstable molecule. It reacts with oxygen or anionic peroxide radiator and produces a peroxide radiator, which directly affects the aging process[13]. At 25μg/mL concentrations of orchid leaf ethanol extracts, the results showed a slight NO production inhibitory trend, but at 50μg/mL and 100μg/mL concentrations, the results showed that NO production was inhibited by 22% and 25%, respectively[Figure 4]. Flavonoids contained in orchid extracts are known to exhibit a wide range of biological activities, including anti-inflammatory and neuroprotective effects. According to Kwon[14,15], flavonoid is believed to have inhibited nitric oxide production due to the activity of flavonoid, a component of orchid extract, and indicates the possibility of its use as an anti-inflammatory agent in orchid extract.

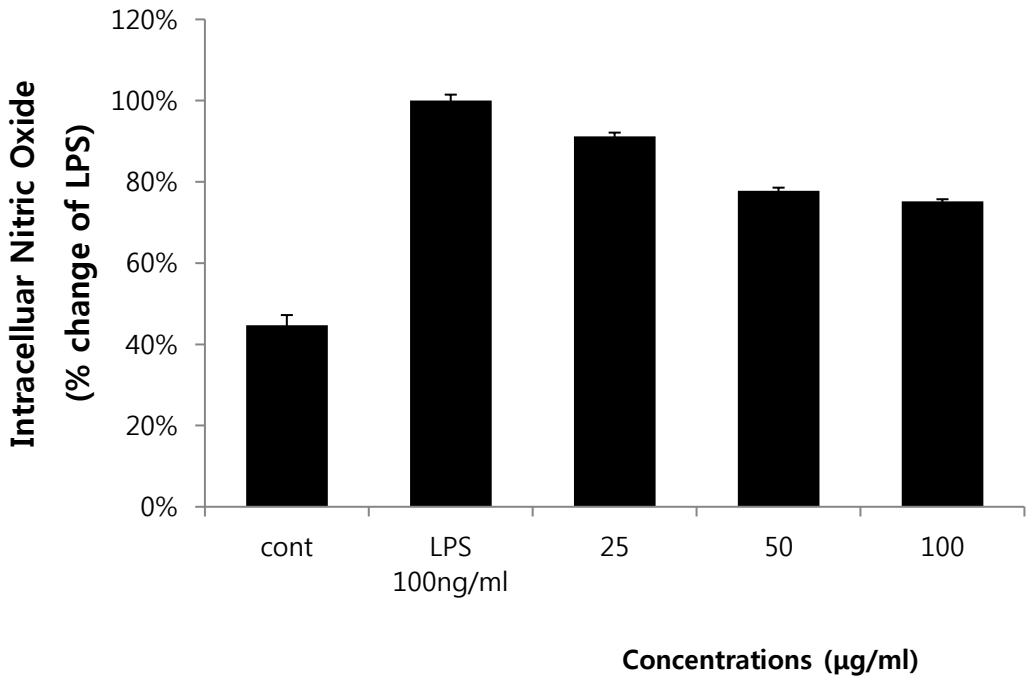

Figure 4. Effects of Orchid leaf ethanol extracts on NO production by 1 μg/mL lipopolysaccharide (LPS) in RAW 264.7 cells. Results are means ± SD from four separate experiments.

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4. CONCLUSION

Orchid has excellent efficacy in the treatment of antioxidants and is widely used in folk remedies, so the possibility of natural antioxidants and anti-inflammatory agents using orchid leaf ethanol extracts belonging to polyphenol compounds is reviewed from the point of view of spices to determine whether they can be cosmetic materials. To observe cytotoxicity, the MTT analysis showed that cytotoxicity of orchid leaf extract was 97% at the highest concentration of 100μg/mL, and thus the orchid leaf ethanol extract was not shown cytotoxic and thus considered a safe substance. Among the various components that reduce oxidative stress, flavonoid glycoside, and others are known as typical antioxidants, and orchids also contain these ingredients. Orchid leaf ethanol extracts is known to have antioxidant properties and was DPPH assay to observe antioxidants and activity on its own. At 25μg/mL concentrations, the results were mild with 5% oxidative stress control, but at 50μg/mL and 100μg/mL, the results were 26% and 43% oxidative stress control, respectively. The cellular oxidation inhibitors inhibited ROS generation at low concentrations of 25μg/mL and inhibited generation of 24% and 29% respectively at concentrations of 50μg/mL and 100μg/mL. These results are believed to be the action of various antioxidant components containing orchid leaf ethanol extracts, which are known to be excellent materials not only for the antioxidant power of the extracts itself but also for the ability to inhibit ROS production within the cell, and can be used as antioxidant and antiaging components in cosmetics. At 25μg/mL concentrations of orchid leaf ethanol extracts, the results showed a slight NO production inhibitory trend, but at 50μg/mL and 100μg/mL concentrations, the results showed that NO production was inhibited by 22% and 25%, respectively. These results suggest that orchid leaf ethanol extracts is not cytotoxic, and it is highly to be used as an anti-aging cosmetic ingredient due to its excellent antioxidant and anti-inflammatory effects.

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