# THE STUDY ON TISSUE CULTURED WILD MOUNTAIN GINSENG(*Panax Ginseng* C.A. Meyer) ADVENTITIOUS ROOTS EXTRACT AS A COSMETIC INGREDIENT

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**Keywords : *Panax Ginseng*, adventitious roots, Dopa auto-oxidation, whitening effect, melanization, anti-wrinkle**

## Summary

Korean ginseng(*Panax Ginseng* C.A. Meyer) known as a oriental miracle drug is an important medicinal plant. Ginseng has been used for geriatric, tonic, stomachic, and aphrodisiac treatments for thousands years. Also, it is an antibiotic and has therapeutic properties against stress and cancer. Ginseng is widely distributed all over the world. Among them, Korean mountain ginseng has the most valuable effect on pharmaceuticals. The roots of mountain ginseng contained several kinds of ginsenosides that have many active functions for the human body. However, the study of mountain ginseng has a limit because the mountain ginseng is very expensive and rare. So, we artificially cultured mountain ginseng adventitious roots using the bioreactor culture system. We induced callus from original mountain ginseng, directly dug up in mountain and aged about one hundred ten years. Separated adventitious roots were precultured in 500ml conical flasks and then, transferred in 20L bioreactors. The adventitious roots of mountain ginseng were harvested after culturing for 40days, dried and then, extracted with several solvents.

In this study, we investigated the whitening effect, anti-wrinkle effect and the safety of tissue cultured adventitious roots extract of mountain ginseng in order to identify the merit as a cosmetic ingredient. Particularly, extract of mountain ginseng adventitious roots showed whitening and anti-wrinkle effects. The inhibitory effect of this extract on the melanogenesis was examined using B-16 melanoma cell. When B-16 melanoma cells were cultured with adventitious root extract, there was a dramatically decrease in melanin contents of B-16 melanoma cell. And we identified this extract inhibited Dopa auto-oxidation significantly. Also, when transformed mouse fibroblast L929 cells were treated with this extract, there was a significant increase in collagen synthesis. The results show significant inhibited melanization and wrinkle without inhibiting cell viability.

## 1. Introduction

Much research has been and continues to be directed to the finding of natural materials, especially herbs capable of whitening the skin. As a result, extracts from various herbs including Morus alba LINNE, Glycyrrhiza uralensis FISCH Glyrhiza inflata BAT, Paeonia lactiflora PALL. P. veitchii LYNCH, Cinnamomum loureirii NEES, Sophora Flavescens Arr., Pueraria thunbergiana(SIEB.et ZUCC) BENTH, Angelica sinensis(OLIV.)DIELS, Paeonia suffruticosa ANDR., Pinellia ternata(THUNB.)BREIT, aloe, etc., are found to be inhibitory of the activity of tyrosinase. However, these herbs do not show definite whitening effects. Additionally, various experiments demonstrate that the herb extracts cannot be used in cosmetically or medicinally effective amounts in the aspects of stability and discoloration of final products. Particularly, only when being used at high concentrations, the herb extracts are, for the most part, inhibitory of the activity of tyrosinase. That is, most of the herb extracts at low concentrations scarcely show inhibitory activity against tyrosinase. Therefore, there remains a need for an improved cosmetic material that is excellent in terms of skin whitening effect and compatibility with other cosmetic materials, with no irritation of the skin.

## 2. Materials and Methods

### 2.1. Preparation of extract from mountain ginseng

- ***Cultivation :*** The adventitious roots were easily induced from the callus on Murashige & Skoog medium(Duchefa) supplemented with 5.0mg/L IBA(Duchefa) and 5% sucrose, and were subcultured in MS liquid medium with the same plant growth regulators.
- ***Extraction :*** After being dried, 1kg of tissue-cultured mountain ginseng adventitious roots was immersed in 30kg of an aqueous 70% ethanol solution and 3kg of 1,3-butyleneglycol, and aged for a sufficient time. Then, the solution was filtered through a filter paper(5C,TOYO), followed by the removal of ethanol from the filtrate at 50-60℃ to obtain an extract.

### 2.2. *In-vitro* cytotoxicity

- ***NR assay :*** 100 ㎕ of the trypsinized transformed mouse fibroblast L929 cells (trypsin 0.05%) which were diluted with DMEM·F'10 containing 2% BCS. $3\times10^3$ cells/well were inoculated into the 96 well and incubated at 37℃ for 24hrs in 5% $CO_2$ incubator. The filtrated testing materials by 0.22㎛ membrane filter were diluted in DMEM·F'10 media at 4 varieties of concentration and 100 ㎕ of each was added into the wells, incubated for 12hrs in 5% $CO_2$ incubator at 37℃. After 0.1ml of neutral red diluted at conc. of 50 ㎍/ml in DMEM·F'10 was added into each well and incubated for 3hrs, the media were removed from each well. After fixing the cells by 1% of formalin, 1% of $CaCl_2$, the accumulated neutral red in the cells was extracted by 1% of acetic acid – 50% of ethanol and it was measured by ELISA reader (Molecular Devices, USA) in dual ranges of 540nm and 630nm. It was plotted in the percentage of each concentration to the control solution(3).

- ***MTT assay:*** The test materials were added and incubated in 5% $CO_2$ incubator at 37℃ for 12hrs by a same procedure of NR assay and 0.01ml of MTT at the conc. of 5mg/ml in PBS was added into well and incubated for 4hrs, and then the residual contents were removed by turning over. To dissolve formazan crystal, Isopropanol containing 0.04N HCl was added and stirred for 20min and it was read by ELISA reader in the dual ranges of 570nm and 630nm(4).

**2.3. Whitening effect**

- ***Inhibition of auto-oxidation by 3,4-dihydroxyphenylalanine(DOPA):*** The solution(5) containing 135U/ml of tyrosinase, 0.03% of DOPA and 0.1M potassium phosphate buffer(pH 6.8) in presence of various concentration of samples were incubated for 1hr at 37℃. Then precipitates were collected by centrifugation. After washing in several times with 6N HCl and distilled water, precipitates dissolved in 2ml of soluene 350. Then absorbance was measured at 475nm(6).
- ***Effect of adventitious roots extract of mountain ginseng on inhibition of melanization. :*** B-16 melanoma cells were cultured in DMEM supplemented with 10% FBS in humidified incubator at 37℃ under 5% $CO_2$ in 6 well plate at density of $2.0\times10^4$ cells/well. After cells were attached, medium was replaced with DMEM containing 10% FBS, 0.2 μM α-MSH, 2mM theophylline after samples addition. After 4days, trypsin was added and cells were collected by centrifugation. Then cell pellets were dried and dissolved in 1N NaOH Melanin synthesis inhibition rates were measured at 490 nm using ELISA reader(7).

**2.4. Anti-wrinkle effect**

- ***Quantitation of collagen synthesis :*** Synthesized collagen from transformed mouse fibroblast L929 was quantitated by the Sirius Red F3BA(BDH,UK). Transformed mouse fibroblast L929 were seeded in 1ml aliquots in 24-well plate at density of $4.0\times10^4$ cells/well in DMEM·F'10 containing 5% FBS, 0.1% BSA and 30 ㎍/㎖ ascorbic acid. After 24hrs incubation at 37℃ the medium was replaced with 1ml DMEM·F'10 containing 2% FBS, 0.1% BSA, 30 ㎍/㎖ ascorbic acid with samples. After 48hrs, cell culture supernanat and cells lysed by rapid freeze thawing, were dried onto the plate. All the plates were incubated at 37℃ for 16hrs(humidified) and then 24hrs at 37℃(dry). The wells were filled with 1ml of 0.1% Sirius Red F3BA in saturated picric acid(w/v) and the samples stained for 1hr at room temperature. The plate were washed five times with 2ml of 10mM HCl for 10s per wash. The collagen bound stain was then extracted with 2ml of 0.1M NaOH for 5min. Absorbance was then read at 540nm in microplate reader(8).

## 3. Results and Discussion

### 3.1. *In-vitro* cytotoxicity

The results are given in Table 1, below.

As apparent from its far higher $NR_{50}$ and $MTT_{50}$ than that of controls, mountain ginseng adventitious roots extract is less toxicity than controls.

| Sample | | $NR_{50}$ | $MTT_{50}$ |
|---|---|---|---|
| Mt. Ginseng Adventitious Roots Extract | | 1↑ | 1↑ |
| controls | Arbutin | 0.59 | 0.61 |
| | Vitamin C | 0.004 | 0.031 |
| | Kojic acid | 0.035 | 0.039 |

Table 1. Comparision of *In-vitro* cytotoxicity

**3.2. Whitening effect**

- ***Inhibition of auto-oxidation by 3,4-dihydroxyphenylalanine(DOPA)***

The results are given in Figure 1, below. The inhibition rate was increased as the concentration of the samples were increased.

The extracts from mountain ginseng adventitious roots show similar inhibition rate compared with that of arbutin. The red ginseng extract shows 7% inhibition rate at 0.1% concentration.

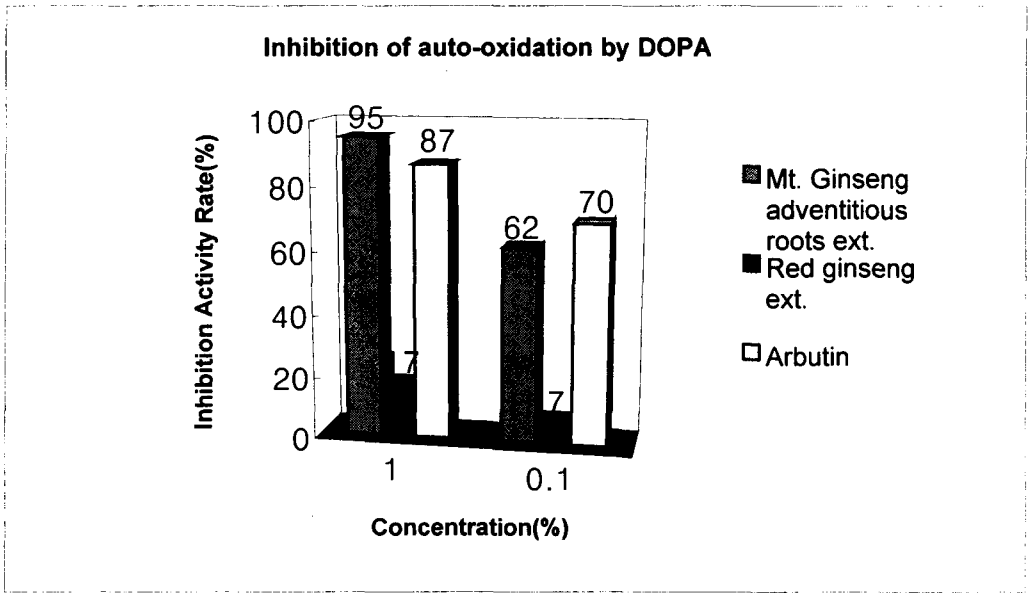


Figure 1. Inhibition of auto-oxidation by DOPA

- ***Effect of adventitious roots extracts of mountain ginseng on inhibition of melanization***

The quantitation of the melanin was analyzed through ELISA to examine the extracts from mountain ginseng adventitious roots for inhibitory activity against melanin synthesis. The results are given in Figure 2 and 3, below.

The extracts from mountain ginseng adventitious roots show excellent whitening effects on the B-16 melanoma cells.

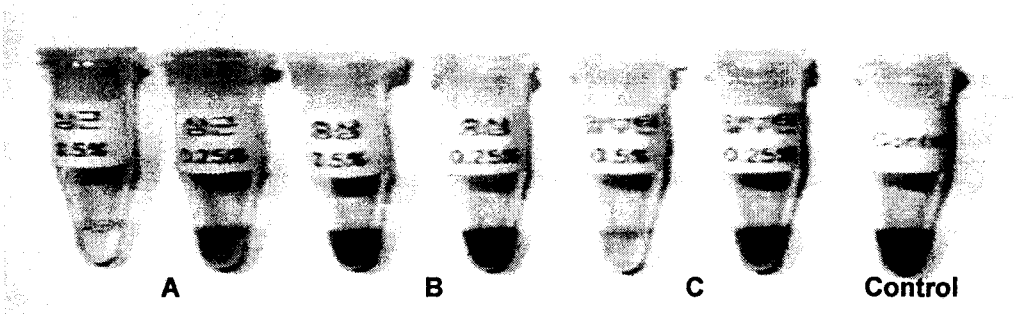


Figure 2. The melanin precipitates of B-16 melanoma

**A** : Mt.Gingeng Adventitious roots ext. (0.5%, 0.25%)

**B** : Red ginseng ext. (0.5%, 0.25%)

**C** : Arbutin (0.5%, 0.25%)

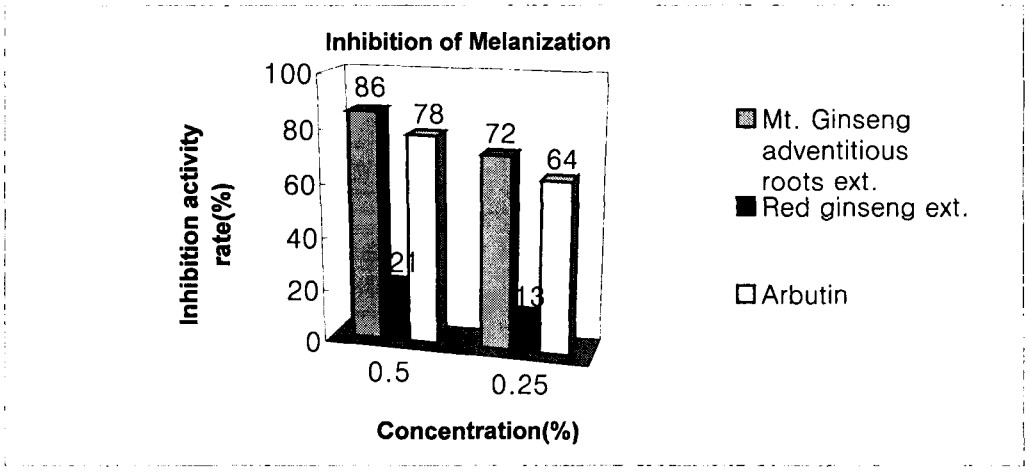


Figure 3. Inhibitory Activity Against Melanin Synthesis

### 3.3. Anti-wrinkle effect

- ***Quantitation of collagen synthesis***

The results are given in Figure 4, below. The extracts from mountain ginseng adventitious roots show better collagen synthesis rate than red ginseng extracts.

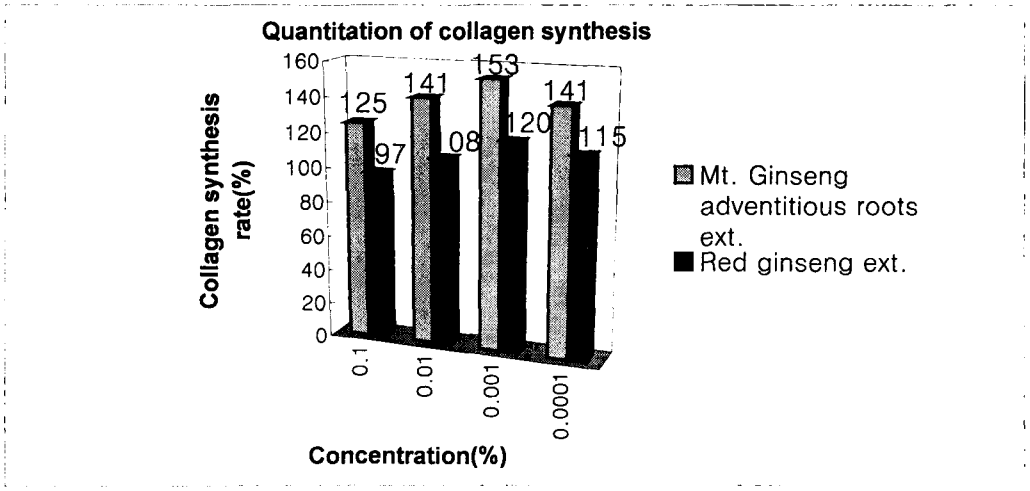


Figure 4. Quantitation of collagen synthesis

## 4. Conclusions

-. In the *in-vitro* cytotoxicity test, extracts from mountain ginseng adventitious roots have less toxicity than Arbutin, Vitamin C, and Kojic acid.

-. In the inhibition of auto-oxidation by DOPA test, the extracts from mountain ginseng adventitious roots show similar inhibition rate compared with that of arbutin.

-. In the inhibition of melanization test, the extracts from mountain ginseng adventitious roots show better inhibition rate than arbutin.

-. In the quantitation of collagen synthesis test, the extracts from mountain ginseng adventitious roots show better collagen synthesis rate than red ginseng extracts.

-. It is very economical and useful to study about the extracts from mountain ginseng adventitious roots which have less toxicity and have good whitening effect. And it is required to further study on which component of this extract has a function on each mechanism.

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