Composition of Phenolic Acids and flavonoids, and Beauty Cosmetic Biological Activities of Korean Mistletoe(Viscum album) Extracts

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Abstract

The present study describes the preliminary evaluation of the composition and beauty cosmetic biological activities of Viscum album L. extracts.

The 8 types of phenolic acids and flavonoids components in both hot water and ethanol extracts were identified. And, flavonoid components were identified 3 types in hot water , and 4 types in ethanol extract. The DPPH radical scavenging activities of hot water and ethanol extracts were measured as 62.55±0.035% and 72.0±0.024% respectively. Tyrosinase inhibitory activity of hot water and ethanol extracts were 63.94±0.0249% and 62.61±0.0124%, respectively. The Inhibition of NO(nitric oxide) production on the hot water and 95% ethanol extracts gradually increased with increasing concentration. In the case of V. album extracted by hot water, Hela, AGS and HT-29 cells showed very good growth in a 60 ㎍/㎖ solution, but cell growth was inhibited in a 100 ㎍/㎖ solution. In the case of V. album extracted by 95% ethanol, Hela cell growth gradually increased with increasing concentration. But AGS and HT-29 cell growth increased in 20 ㎍/㎖. On the other hand, cell growth tended to decrease in higher than 60 ㎍/㎖ concentrations. The microbial growths of S. aureus, E. coli, P. aeruginosa and S. typhimurium tested with hot water extract increased dependent on concentration. But growth of K. pneumoniae bacteria has increased until 300 ㎍/㎖, but antimicrobial activity started to appear at higher than 300 ㎍/㎖ concentrations. In these case of 95% ethanol extract, microbial growth of 5 strains growth depended on the concentration of the extract, and cell growth not inhibited in treated concentration(1,500 ㎍/㎖) of this study.

Keywords: *anti-inflammatory effect, antioxidant, anti-tumor, phenolic acids and flavonoids, tyrosinase activity, antimicrobial effect, V. album*

1. Introduction

Korean mistletoe(Viscum album L.) includes three widely distributed subspecies that differ in host specificity(Ball 1993; Böhling *et al.*, 2002). Viscum includes approximately 100 species most of them in Africa and Madagascar and a smaller number in southern Asia(Zuber, 2004). Mistletoe is a common semiparasitic plant which grows on deciduous trees all over the

world. In the Far East, mistletoes has been taken as tea or decoction as a traditional therapy for ataralgesia, lumbago, hypertension, tumor *etc.*,(Huang, 1993). Also, bacterially fermented aqueous extracts of mistletoe have a cytostatic effect on animal tumor cell in culture(Khwaja *et al.*, 1983). Moreover, many investigators(Kuttann *et al.*, 1990; Mueller and Anderer, 1990; Kuttan and Kuttan, 1992) have demonstrated that the extract of V. album L. augmented antitumor effect by enhancing the cytotoxic activity of NK cells, and has a antimicrobial effect on some microorganisms(Costa, 2010). Korean mistletoe is also used as a traditional herb for the treatment of cancer, cadiovascular disease, and arthrosis. The identified bioactive components of mistletoe are lectin, alkaloids, viscotoxins, steroids, triterpenes, flavonoides, and polysaccharides(Samuelsson, 1974). Also, in Korea, mistletoes were used to treat trachyarrhythmia(Wu *et al.*, 1995), acute myocardial infraction(Zhu, 1984, Zhu *et al.*, 1985, Zee Cheng, 1997) and schizopherenia(Okuda *et al.*, 1987). In addition, Korean mistletoes is a potent immunoadjuvant, enhancing cellular and humoral immune responses(Yoon *et al.*, 2001). It has also been that an extract of Korean mistletoe had antitumor activity, inhibiting tumor metastasis in mice, and its anti-tumor activity was related to suppression of tumor growth and tumor-induced angiogenesis(Yoon *et al.*, 1995), as well as enhancement of NK cell activity(Yoon *et al.*, 1998) and cytotoxic and immunological effects(Lyu and Park, 2007). In this study, we investigated the composition of phenolic acids and flavonoids, and beauty cosmetic biological activities and possible benificial beauty cosmetic ingredient potency of V. album extracts on the polyphenol and flavonoid contents, antioxidant, tyrosinase activity, anti-inflammatory effect, anti-tumor and antimicrobial effect.

2. Material and Methods

All printed material, including text, illustrations, and charts, must be kept within the parameters of the 8 15/16-inch (53.75 picas) column length and 5 15/16-inch (36 picas) column width. Please do not write or print outside of the column parameters. Margins are 1 5/16 of an inch on the sides (8 picas), 7/8 of an inch on the top (5.5 picas), and 1 3/16 of an inch on the bottom (7 picas).

2.1. Plant Material and Extraction

Viscum album was collected around the Jangan mountain Jangsu Jenrabukdo Korea in January 2009. Each sample was separately prepared by freeze drying and grinding and finally stored at − 70℃ conical tubes. The resulting powder of each plant(10g) was extracted in 100 ㎖ of hot water and 95% ethanol using a soxhlet extractor for 3hr at 100℃ and 80℃, respectively. The solution obtained from each powder was concentrated under vacuum to give a crude dried sample.

2.3. DPPH Free Radical Scavenging Activity

An assay for DPPH(1,1-diphenyl-2-picrydrazyl) free radical scavenging potential was performed as method. The sample solution(200 ㎕) mixed with 100mM Tris-HCl(pH 7.4) 800ul and afterwards added to a 500uM DPPH solution 1ml dissolved in methanol. After mixing incubation at room temperature for 20min, absorbance was then measured at 517nm by UV-VIS spectrophotometer. BHA and BHT were used as positive controls and calculated as: Scavenging activity (%) = 〔(A0-A1)/A0)〕 X 100, where A0 was the absorbance of the control and A1 was the absorbance in the presence of the test compound.

2.4. Tyrosinase Inhibitory Activity Assay

Mushroom tyrosinase and L-tyrosinase were purchased from Sigma(St. Louis, MO, U.S.A). Assay were performed as method. The reaction mixtures, consisting of 200 ㎕ of 0.175M phosphate buffer(pH 6.8), 200 ㎕ of 5mM L-DOPA, 100 ㎕ of mushroom tyrosinase(2,000 U/㎖) and 500 ㎕ of sample, were mixed in ephendrop tube and pre-incubated at 35℃ for 2min. Optical densities were measured at 475nm. Tyrosinase inhibition percent was calculated as follows: % inhibition = (1-(S-B/C)) X 100, where, S=O.D. tyrosinase, sample and L-DOPA; B=O.D. without the tyrosinase; C=O.D. without the test sample; all O.Ds were determined at 475nm.

2.5. Statistical Analysis

The data are expressed as mean±standard deviation(SD) for at least three independent determinations in triplicate or quadruplicate for each experimental point. Differences among all sample means were determined by analysis of variance(ANOVA) using SPSS 12.0 for Microsoft and were considered significant at $p<.05$.

3. Results and Discussion

3.1. DPPH Radical Scavenging Activity

The DPPH radical scavenging activities of hot water and ethanol extract of V. album are presented in Table 1. The radical scavenging activities against control, BHA and BHT were 83.91±0.045% and 83.86±0.032%, respectively. But experimental results was showed 62.55±0.035% in hot water, and 72.0±0.024% in ethanol extract.

Table 1. The DPPH Radical Scavenging Activity of V. Album Extracts

Solvents	DPPH radical scavenging activity(%)
hot water	62.55±0.035
95% ethanol	72.00±0.024
BHA	83.91±0.045
BHT	83.86±0.032

* BHT and BHA are butylated hydroxy anisole and butylated hydroxy toluene, respectively. In the present results, radical scavenging activity of V. album extracts was somewhat lower than that of control groups. Also, the DPPH radical scavenging activity changed according to the extract solvent used. The antioxidant capacity of natural antioxidants was due to the termination of the free radical reaction(Shimada *et al.*, 1992). In a similar study, Onay *et al.*, (2006) reported that radical scavenging activity(95.12±2.37% DPPH inhibition) of no. 4 V. album methanol extract was found to be even higher than reference antioxidants. Also, Kim *et al.*, (2010) reported that Korean mistletoe lectin showed DPPH radical scavenging activity with an IC50 value of 42.6 ㎍/㎖.

3.2. Composition and Contents (mg%) of Phenolic Acids and Flavonoids

The phenolic compounds act as electron donors and hence protect living cells and tissues from free radical mediated oxidative stress such as aging and human degenerative disease(Finkel and Holbrook, 2000; Urquiaga and Leighton, 2000). Flavonoids, also known

as nature's tender drugs, possess various biological and pharmacological, antiviral, anti-inflammatory, immunomodulatory, and antithrombotic activities(Havsteen, 1983). Also, natural antioxidants can protect the human body from free radicals and retard the progress of many cronic diseases as well as lipid oxidative rancidity in food, cosmetics and pharmaceutical materials(Kinesella *et al.*, 1993; Lai *et al.*, 2001).

The proximate compositions of phenolic acids and flavonoids of V. album hot and ethanol extracts are presented in Table 2. The composition and content of phenolic acids of hot water extract was grater, in order of 175.05 ㎎%, 4-hydroxybenzoic acid, 128.37 ㎎% ρ-coumaric acid, 119.76 ㎎% syringic acid, respectively. And, in the ethanol extracts, 450.04 ㎎% syringic acid, 348.03 ㎎% ρ-coumaric acid, 279.63 ㎎% 4-hydroxybenzoic acid, respectively. Therefore, 8 types of phenolic acid and flavonoids component in both hot water and ethanol extracts were identified. In the composition and content of flavonoids of hot water extract, quercertin was topped with 264.13 ㎎%, however, isorhamnetin was not detected, and 3 types of flavonoides composition were identified. But composition flavonoids in the ethanol extract was grater, in order of 425.71 ㎎% luteolin, 391.86 ㎎% quecertin, 181.83 ㎎% kaempferol, 135.02 ㎎% isorhamnetin, respectively. And 4 types of flavonoids composition in ethanol extracts were identified.

Table 2. Composition and Contents(㎎%) of Phenolic Acids and Flavonoids Isolated from V. Album Extracts

Compositions		Hot water	95% ethanol
Phenolic acids	ferulic acid	79.09	210.36
	4-hydroxybenzoic acid	175.05	279.63
	ρ-coumaric acid	128.37	348.03
	rosmarinic acid	47.15	136.85
	trans-m-coumaric acid	44.10	249.25
	sinapic acid	37.31	168.93
	syringic acid	119.76	450.04
	trans-cinnamic acid	17.43	58.44
Flavonoids	kaempferol	7.59	181.83
	luteolin	5.43	425.71
	quecertin	264.13	391.86
	isorhamnetin	ND	135.02

* ND : not detected

3.3. Tyrosinase Inhibition

Tyrosinase inhibitory activities of hot water and ethanol extract of V. album are presented in Table 3. Tyrosinase inhibitory activity of hot water and ethanol extracts were 63.94±0.0249% and 62.61±0.0124%, respectively. Therefore, these results also indicated that extracts of V. album can be used as a natural ingredients with whitening effects in cosmetics, toothpaste etc.

Table 3. Tyrosinase Inhibitory Effect of Extracts of V. Album

Solvents	Inhibitory effect(%)
hot water	63.94±0.0249
95% ethanol	62.61±0.0124
vitamin C	42.5±0.1217

* Tyrosinase activity was measured by extracted solution from 10g plant power in 100 ㎖ hot water and 95% ethanol. Vitamin C was treated with 100 ㎍.

3.4. Effects of Antitumor Activity

The results of antitumor effect against Hela, AGS and HT-29 cell lines are shown in Figure 1. In the case of V. album extracted by hot water, Hela, AGS and HT-29 cells grew very well in 60 ㎍/㎖, but cell growth was inhibited in 100 ㎍/㎖. In these results, AGS cell growth was inhibited by 9.51%, and HT-29 cell by 10.62% compared to the control group. In the case of V. album extracted by ethanol, Hela cell growth gradually increased with increasing concentration. However, AGS and HT-29 cell growth showed an increase at 20 ㎍/㎖. On the other hand, cell growth tended to decreasing at higher 60 ㎍/㎖ concentration.

The major cytotoxic components of mistletoe are lectins, viscotoxins and alkaloids, and among these lectins are the most cytotoxic(Park *et al.*, 1999). And, lectin I is the most important component in the crude extract according to many researchers(Olsners *et al.*, 1982; Doser *et al.*, 1989). Khwaja *et al.*,(1986) also showed that mistletoe lectin is more cytotoxic than viscotoxins and alkaloids in their system using leukemia L1210. But in the heat-treated(heating for 30 min) extract, the denaturation of lectin I seemed to cause a marked decrease in the cytotoxicity(Park *et al.*, 1997). In these results, cytotoxicity on the cells was similar to Park *et al.*,(1999) results.

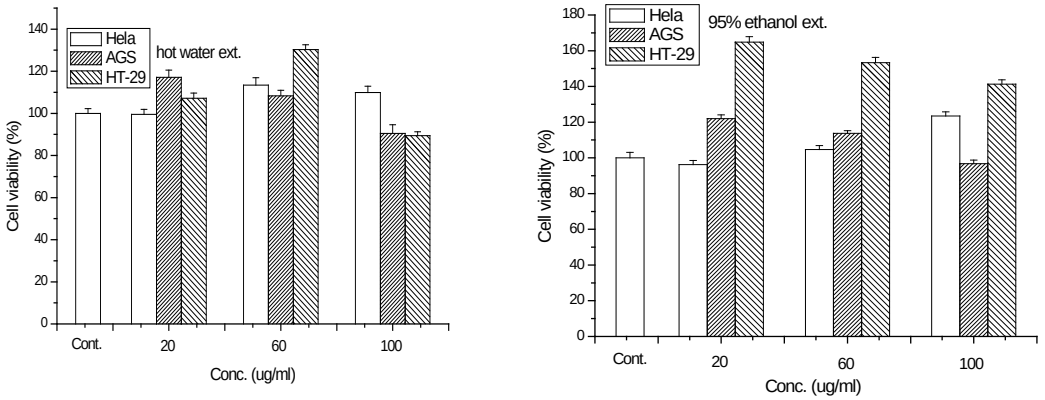

Figure 1. The Effects of Hot Water and 95%Ethanol Extracts of V. Album on the Viability of Hela, AGS and HT-29 Cells. Cells were treated with 0, 20, 60 and 100ug/ml for 48hr, and Cell Viability was determined by MTT Assay as described in Material and Methods. Each Bar represent Mean ± Standard Deviation. Statistically Significant Value compared with Control Group(p <.001)

3.5. Inhibitory Effect of NO Production

Anti-inflammatory activity of extracts was measured on Lipopolysaccharide(LPS)-induced RAW 264.7 cells. The level of nitric oxide product is an important reflector during the inflammatory progress. Also, the NO radical is known to play a central role in inflammatory and chronic immune reactions. Excessive NO production from activated macrophages has also been observed(Wang, 2006).

Figure 1 shows the results of NO production in RAW 264.7 cell after the treatment with 0-100 ㎍/㎖ of V. album extracts. The results showed that the inhibition of NO production on the hot water and ethanol extracts gradually increased with increasing concentration. Inhibition rate of NO production in hot water and ethanol extract was about 13.04%, and about 43.36% at 80 ㎍/㎖ concentration, respectively. On the other hand, NO production in samples treated with 100 ㎍ / ㎖ of hot water and ethanol extracts showed some increase(Figure 2). But, Kim *et al.*, (2010) report that NO scavenging effects of Korean mistelote lectin on radical induced oxidative stress was seen with 26.0%, 49.6% and 57.2% at concentrations of 10, 50, and 500 ㎍/㎖, respectively.

Consequently, inhibitory effects of NO production appeared at less than 80 ㎍/㎖ by hot water extract, and 100 ㎍/㎖ by ethanol extract.

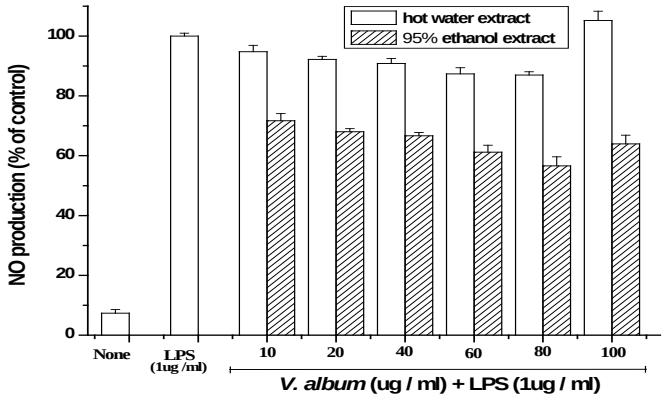

Figure 2. Effects of Hot Water and 95% Ethanol Extracts of V. Album on Nitric Oxide Production in LPS-induced RAW264.7 Mouse Macrophages. Cells were treated with various Concentration(0, 10, 20, 40, 60, 80 and 100 ㎍/㎖), and 1ug/ml of LPS for 24hr. NO Production measured by the Griess Reagent System as described in Material and Methods. Control Sample indicates the LPS-stimulated Cells. Each Bar represent Mean ± S.D. Statistically Significant Value compared with Control Group(p <.001)

3.6. Antimicrobial Activity

The results of antimicrobial activities against 5 microbial strains appear in Figure 3. In these results, microbial growths of S. aureus, E. coli, P. aeruginosa and S. typhimurium tested with heat water extract increased dependent on concentration. But growth of K. pneumoniae bacteria increased up 300ug/ml, and antimicrobial activity appeared at higher than 300 ㎍/㎖. In the case of ethanol extract, microbial growth of all 5 strains occurred regardless of the

concentration of the extract, that is, all bacteria growth does not demonstare any anti-microbial activity with the ethanol extract.

In a similar study, Lee *et al.*, (2012) reported that antimicrobial activity of C. longa extract was observed on the same bacteria in this study. In these result, antimicrobial activity of S. pyrogenes tested with hot water extract appeared at higher than 1,000 ㎍/㎖, and there no antimicrobial activity in other bacteria, S. aureus, E. coli, P. aeruginosa and S. typhimurium, but in ethanol extract, antimicrobial activity appeared in tested with all bacteria. Also, Costa *et al.*, (2010) reported that antimicrobial activity of Phthinus pyrifolia leaf lectin(PpyLL) was observed on the bacteria S. Epidermidis, B. Subtilis, K. Pneumoniae, F. Lateritium and R. Solani. In these results, antimicrobial activity was similar to Costa *et al.*,(2010) results.

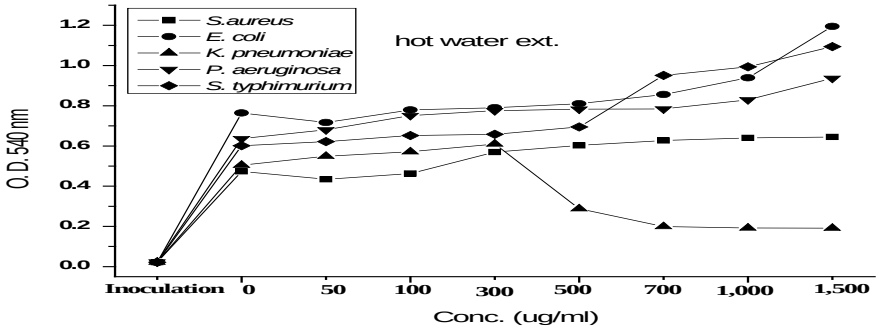

Figure. 3. Antimicrobial activities of hot water and 95% ethanol extracts of V. album against 5 microbial strains. Microorganisms were treated with 0, 50, 100, 300, 500, 700, 1,000 and 1,500ug/ml for 24hr at 37℃ cultured in nutrient broth and tryptic soy broth. Bacteria growth was determined by spectophtometrically at 540nm

Therefore, the antimicrobial activity of V. alum hot water extract was not shown against used most of bacteria in treated with concentration of this study, and was more effective for gram negative than for gram positive species of bacteria. Consequently, in these results, we think that, V. album hot water and ethanol extracts was found antioxidant, whitening effect, anti-inflammatory activity, antitumor and some antimicrobial activity.

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