Korean Society for Biotechnology and Bioengineering Journal 31(2): 126-134 (2016)
http://dx.doi.org/10.7841/ksbbj.2016.31.2.126 ISSN 1225-7117 / eISSN 2288-8268

연구논문

# 바나나 잎 추출물의 주름개선 효과

유대성[1], 장수진[1], 박윤정[1], 김승회[2], 황형서[3]*

# Antiaging Effects of *Musa sapientum L.* (Banana) Leaf Extract

**Dae Sung Yoo[1], Su Jin Jang[1], Yun Jeong Park[1], Seung Hoi Kim[2], and Hyung-seo Hwang[3]***

Received: 11 February 2016 / Revised: 14 June 2016 / Accepted: 20 June 2016


**Abstract:** To examine the possibility of using *Musa sapientum* L. (Banana) leaf extract as a cosmetic raw material, banana leaves grown in Jeju Island were extracted with 70% ethanol. Polysaccharides present in banana leaf extract were discarded by precipitation with cold ethanol. Polysaccharide-discarded banana leaf extract promoted procollagen and *COL1A1* gene expression, but inhibited matrix metalloproteinase (*MMP)-1* and *MMP-2* gene expression in human skin fibroblasts when examined by real-time reverse-transcription polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA). The active compound in banana leaves was identified by fractionation with various solvents. The chloroform fraction showed the highest anti-wrinkle efficacy and the active compound of chloroform fraction was identified as corosolic acid by NMR, FT-IR, EA, and HPLC-MS. In addition, banana leaf extract showed anti-oxidative efficacy with an IC50 value of 67.91 ppm, as determined by DPPH free radical scavenging assay. Finally, the anti-wrinkle efficacy of banana leaf extract-containing cream was confirmed by clinical tests. Based on these results, banana leaves could have an application as a cosmetic raw material with anti-wrinkle efficacy.

**Keywords:** *Musa sapientum L.* leaves, Corosolic acid, Anti-aging, Anti-wrinkle

[1]주식회사 에이씨티 기술연구소
[1]ACT R&D CENTER 7F, 114-6 Central Town-ro, Yeongtong-gu, Suwon-si, Gyeonggi-do, 16506, Korea
Tel: +82-31-215-4893, Fax: +82-31-215-4851
e-mail: dsyoo@actcos.com

[2]단국대학교 자연과학대학 화학과
[2]Department of Chemistry, Dankook University, Cheonan 31116, Korea

[3]세명대학교 한방바이오융합과학부 한방화장품과학과
[3]School of Integrated Oriental Medical Bioscience, Semyung University, Jecheon 27136, Korea
Tel: +82-43-649-1618, Fax: +82-43-649-1730
e-mail: hshwang@semyung.ac.kr

## 1. INTRODUCTION

Aging is associated with changes resulting from decreased physical and psychological function. Typically, the signs of skin aging include xeroderma, changes in skin tone and elasticity, vasodilation, and wrinkles. Wrinkles are caused by intrinsic aging and photo-aging.

Wrinkles are influenced by age, external environment, and UV irradiation. Typical age-related skin damage increases skin stress and stratum corneum hypertrophy and decreases stratum corneum moisture, elasticity, and flexibility. UV-induced skin damage is characterized by skin inflammation, DNA damage, pigmentation, and in severe cases, skin cancer [1,2]. The mechanism of photo-aging involves decreased collagen synthesis and over-expression of matrix metalloproteinases (MMPs) induced by UV irradiation, which results in aging processes such as wrinkle formation [3].

Collagen is the most abundant protein in the body, accounting for approximately 25% of the entire body protein content. Bundles of collagen molecules called “collagen fibers” have a distinctive triple helix structure that provides excellent strength and elasticity. Collagen fibers are the major component of the extracellular matrix (ECM), supporting most of the connective

tissue in animals. Strong, mature type I collagen fibers are formed from triple-stranded, type I procollagen molecules by extracellular enzymatic processes. Type I procollagen molecules contain two pro-alpha-1 chains and one pro-alpha-2 chain that are encoded by *COL1A1* (collagen, type I, alpha 1) and *COL1A2* (collagen, type I, alpha 2), respectively [4].

*MMPs* are enzymes that are directly responsible for the degradation of ECM components such as collagen, gelatin, and elastin. *MMP-1*, an interstitial collagenase, belongs to a subfamily of *MMPs* that can specifically degrade the collagen triple helix. *MMP-2* is a gelatinase that degrades denatured collagen, gelatin, and elastin. UV irradiation induces the degradation of collagen by increasing the production of MMPs in the skin. Therefore, the regulation of *MMP* activity may protect the skin from damage caused by UV irradiation.

Oxidative stress induced by free radicals is also associated with skin aging. Free radicals are defined as molecules containing one or more unpaired electrons that are capable of independent existence. These unstable states make them highly reactive, thereby increasing their generation via initiation of chain reactions. Finally, they can alter protein function, induce gene damage, and promote disease progression and aging [5]. Free radicals affect the skin in a number of ways. They alter lipids in cellular membranes, gradually affecting the cell structure, and control the passage of other molecules. Further, they genetically modify cells resulting in disease or make the skin more susceptible to premature aging. Free radicals also lead to cross-linkage of collagen fibers in the dermal component of the skin. This results in the formation of wrinkles and sagging accompanied by loss of skin tone.

*Musa sapientum L.* (Banana) is mainly grown in tropical and subtropical countries and is widely used for its nutritional value worldwide. The fruit of the plant is used to treat many diseases in humans such as diarrhea, intestinal lesions caused by ulcerative colitis, diabetes, uremia, and hypertension. Since only the fruit is used for medicinal purpose, the other parts of the plant are discarded.

In this study, the anti-aging effects of banana leaf extract were assessed by evaluating its anti-oxidative and anti-wrinkle activity *in vitro*. Additionally, the anti-wrinkle efficacy and safety were assessed *in vivo* on human skin.

## 2. MATERIALS AND METHODS

### 2.1. Banana leaves

Banana leaves were purchased from a certified eco-friendly banana farm in Jeju Island, Korea. The banana leaves were washed with tap water, dried, and stored protected from moisture until further use.

### 2.2. Preparation of banana leaf extract

Dried banana leaves were extracted with water at 80°C or 70% aqueous ethanol at 50~60°C for 4 hours. The extracts were concentrated to 1/10-fold using a vacuum evaporator and addition of 10 folds of cold ethanol removed polysaccharide from the extract. Polysaccharide-discarded banana extract was concentrated using a vacuum evaporator and then lyophilized. The powdered extracts were dissolved in DMSO and diluted.

### 2.3. Cell culture

Human fibroblast cells, Hs68 were purchased from ATCC (American Type Culture Collection) and cultured in Dulbecco's modified eagle medium (DMEM) with 10% fetal bovine serum (FBS) and 100 U/ml penicillin/streptomycin at 37°C under 5% $CO_2$. FBS, penicillin/streptomycin, and trypsin-EDTA were provided by Gibco (Massachusetts, USA).

### 2.4. Cell viability assay

Cell viability and the extent of proliferation were assessed by conventional MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolum bromide] assays. Briefly, cells ($5\times10^4$ cells/well) were incubated with test material for the indicated times and further incubated with MTT solution (0.5 mg/mL) for an additional 3 hours at 37°C. The absorbance of the samples was measured at 490 nm using a microplate reader (Molecular Devices Corp., CA, USA).

### 2.5. Enzyme-linked immunosorbent assay (ELISA)

To determine procollagen expression levels, Procollagen Type C-peptide (PIP) EIA Kit (TaKaRa, Japan) was used in accordance with the manufacturer's instructions. Briefly, cells ($1\times10^5$ cells/well) were incubated with test material for 24 hours and harvest the supernatant. It is necessary to dilute the supernatant about 5~10 fold. Transfer 100 mL of antibody-POD conjugate solution into one well, and subsequently add 20 mL sample or standard. Seal the microtiter plate and stand for 3 hours at 37°C. After 3 hours, remove contents by suction and wash the wells 4 times with 400 mL of PBS. Between the separate washing steps empty out the microtiter plate and vigorously tap onto paper towel, especially after the last washing. Add the 100 mL of substrate solution into each well and incubate at room temperature (20~30°C) for 15 minutes, and subsequently add 100 mL of stop solution into each well in same order as for substrate. The absorbance of the samples was measured at 450 nm using a microplate reader (Molecular Devices Corp., CA, USA).

**Table 1.** The scores for level of wrinkle using *In vivo* clinical test

| Level of wrinkle | Score |
|---|---|
| None | 0 |
| None/mild | 1 |
| Mild | 2 |
| Mild/moderate | 3 |
| Moderate | 4 |
| Moderate/Severe | 5 |
| Severe | 6 |
| Very severe | 7 |

### 2.6. Real-time reverse-transcription polymerase chain reaction (Real time RT-PCR)

Effects of banana leaf extract on the promotion of wrinkle-related gene expression were investigated with RT-PCR. Total RNA was isolated from Hs68 cells with TRIzol Reagent (Gibco, NY, USA), in accordance with the manufacturer's instructions. Total RNA was stored at -70°C until use. Analysis of mRNA was also performed using Real time RT-PCR with LightCycler® 96 (Roche, Mannheim, Germany). PCR was carried out using SYBR Green PCR Master Mix (Applied Biosystems, Massachusetts, USA) as described [6]. The results were expressed as the ratio of target mRNA expression to GAPDH mRNA expression. The primers (Bioneer, Daejeon, Korea) used for Real time RT-PCR are listed in Table 1.

### 2.7. HPLC analysis

Chloroform fraction of banana leaf extract was subjected to a RP-C18 column (250 mm × 4.6 mm; Kanto, Japan). The HPLC system was used Waters 2424 alliance separation module (Waters, USA). The chloroform fraction was chromatographed on a column eluted with 7:3 (v/v) of acetonitrile and 0.1% phosphoric acid at a flow rate of 1.0 mL/min and monitored at 203 nm.

### 2.8. NMR, IR, EA, and MS analysis

To determine the qualitative components and the relative proportion of hydrogen or carbon in base materials, NMR (Nuclear Magnetic Resonance) system were used (Ascend 500, Bruker, Germany). The IR (Infrared Spectroscopy) system (Frontire, PerkinElmer, USA) were used for checking the functional group in base materials. The EA (Elemental Analysis) system (FLASH EA-2000, Thermo scientific, USA) were used for identifying the presence or absence of various chemical elements; nitrogen, carbon, hydrogen and sulphur in base materials. The MS (Mass Spectrometer) analysis system (Clarus 600 T, PerkinElmer, USA) were used for measuring molecular weight of base materials. All of these systems were performed at common equipment center of Dankook University.

### 2.9. DPPH free radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activities were evaluated using the method of Blois (1958), with minor modifications. DPPH solution (0.1 mM in ethanol) was added to the same volume of sample solution, allowed to react for 10 min at room temperature, and the optical density was then measured at 565 nm using a microplate reader (Molecular Devices Corp).

### 2.10. *In vivo* clinical test

*In vivo* clinical testing was conducted with 23 healthy female volunteers aged between 30 to 65 years. Volunteers used either 0.04% adenosine-containing cream (positive control) or banana extract cream-containing 0.75% corosolic acid. (Briefly, to make the 0.75% corosolic acid containing cream, we made the extract included high-content of corosolic acid by using purification process, and then we made liposome included 25% corosolic acid using the purified extract. Finally, we added 3% of liposome into the cream formulation; the final concentration of corosolic acid in cream is 0.75%). This was applied around their eyes (crow's feet) twice a day for 8 weeks. After the 8$^{th}$ week, their crow's feet areas were measured with PRIMOS Lite (field of view 18×13, FRMesstechnik GmbH, Germany) and Robo Skin Analyzer CS50 (inforward, Inc., Japan) in accordance with manufacturers' instructions. Dermatologists also evaluated the wrinkles using a global photo damage score with double blind test. The scores used are represented in following Table 1.

### 2.11. Statistical analysis

Data were analyzed using paired *t*-test by using SPSS 17.0 for windows (SPSS, Chicago IL, USA). Differences were considered significant at $p<0.05$.

## 3. RESULTS AND DISCUSSIONS

Extrinsic and intrinsic skin aging commonly increase wrinkling, sagging, and decreased elasticity [7]. Extrinsic aging is generally related to photo-aging and is caused by repeated exposure to UV light. UV irradiation induces the synthesis of MMPs in fibroblasts. MMPs, a family of zinc-dependent endoproteinases, play a key role in remodeling ECM structures during wound healing [8], dermal photo-aging [9], and several pathologies such as tumorigenesis [10]. For example, MMP-1 initiates the cleavage of fibrillar collagen (Type I and III in the skin) at a single site within its central triple helix. Once cleaved by MMP-1, collagen is further degraded by other MMPs owing to their upregulated activities [11].

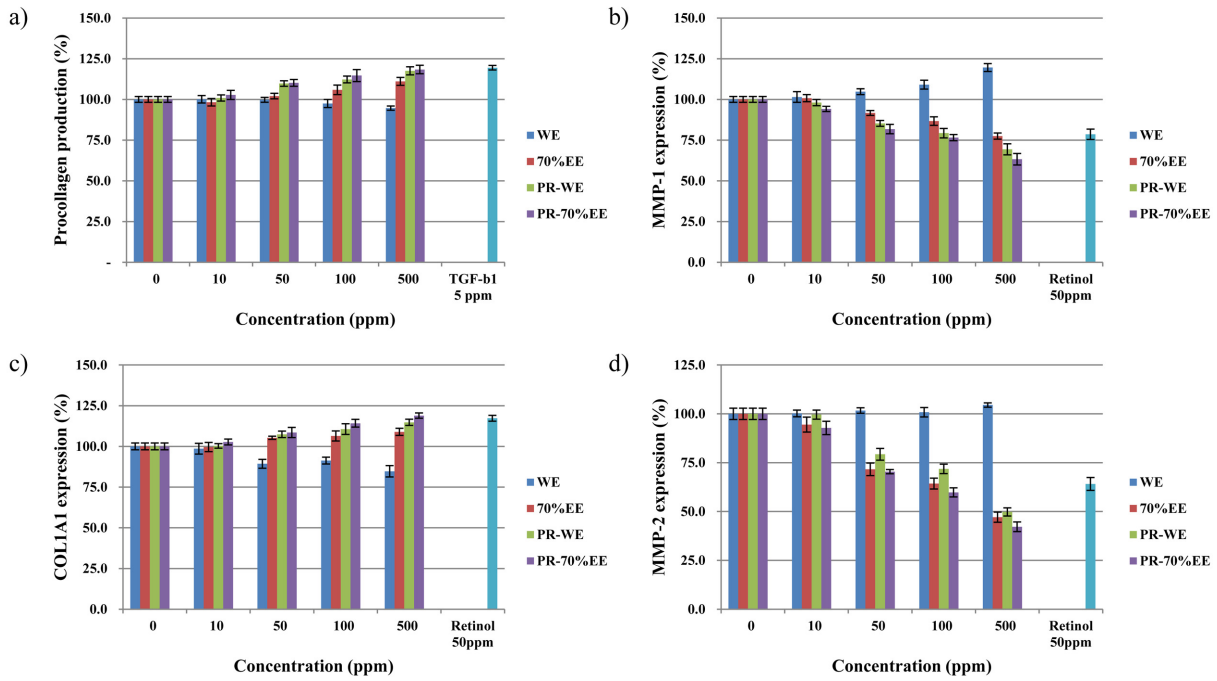


**Fig. 1.** Effect of *Musa sapientum* L. (Banana) leaves extracts on the production of wrinkle mediators. (a) Cell viability and levels of procollagen were determined by ELISA in culture supernatants of Hs68 cells treated with banana leaves extracts for 24 h. (b-d) Effect of Banana leaves extracts on the expression of wrinkle-mediated genes. Levels of mRNAs expression were determined by Real time RT-PCR. (*WE, Water extract; EE, Ethanol extract; PR: polysaccharide-removed. Error bar indicate standard deviation.

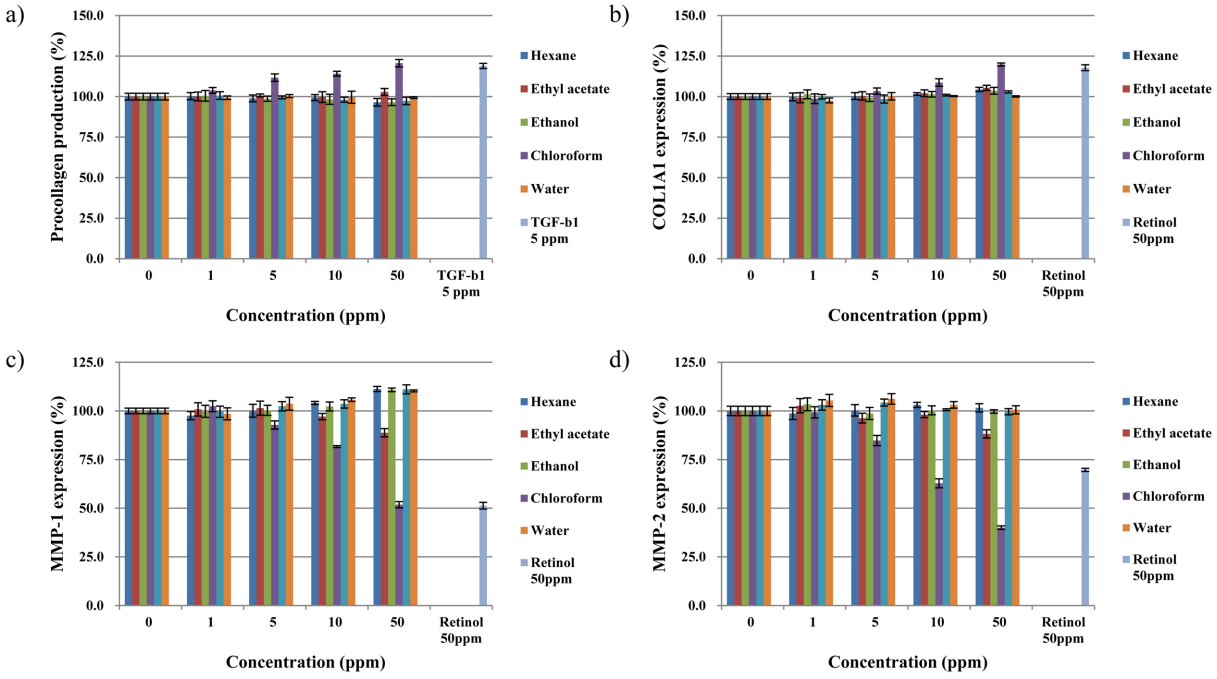


**Fig. 2.** Effect of each banana leaves extracts on the wrinkle-formation related gene expression. After banana leaves were extracted with 70% ethanol, polysaccharides were removed as described in material and methods. Polysaccharide removed extract was fractionated with various solvent. (a) Cell viability and levels of procollagen were determined by ELISA in culture supernatants of Hs68 cells treated with these samples for 24 h. (b-d) Effect of these samples on the expression of wrinkle-mediated genes. Levels of mRNAs were determined by Real time RT-PCR. Error bar indicate standard deviation.

The expression of COL1A1, MMP-1, and MMP-2 genes and procollagen involved in photo-aging and the subsequent formation of wrinkles were examined by RT-PCR and ELISA in human skin fibroblasts treated with banana leaf extract. As shown in Fig. 1, banana leaf extract up-regulated procollagen and COL1A1 expression and downregulated MMP-1 and MMP-2 expression. Notably, the polysaccharide-removed 70% ethanol extract showed the highest upregulating effect on procollagen and COL1A1 expression, and the highest downregulating effect on MMP-1 and MMP-2. Thus, polysaccharide-removed banana leaf extract showed excellent antiwrinkle efficacy.

Polysaccharides are commonly found in nature as plant-building materials (cellulose). They also serve highly specialized functions such as protective coating for bacteria and other cells. However, an inherent dark color presents a challenge for its use as a cosmetic ingredient. Therefore, it is necessary to discard the polysaccharides in plant material in order to use it in cosmetics. Additionally, skin absorption rate could be increased by discarding this polysaccharide.

Polysaccharide-removed banana leaf extract prepared with 70% ethanol was fractionated with various solvents such as hexane, chloroform, ethyl acetate, methanol, ethanol, and water, and COL1A1, MMP-1, and MMP-2 gene expression of each fraction was compared. The chloroform fraction showed the highest upregulating effect on procollagen and COL1A1 and

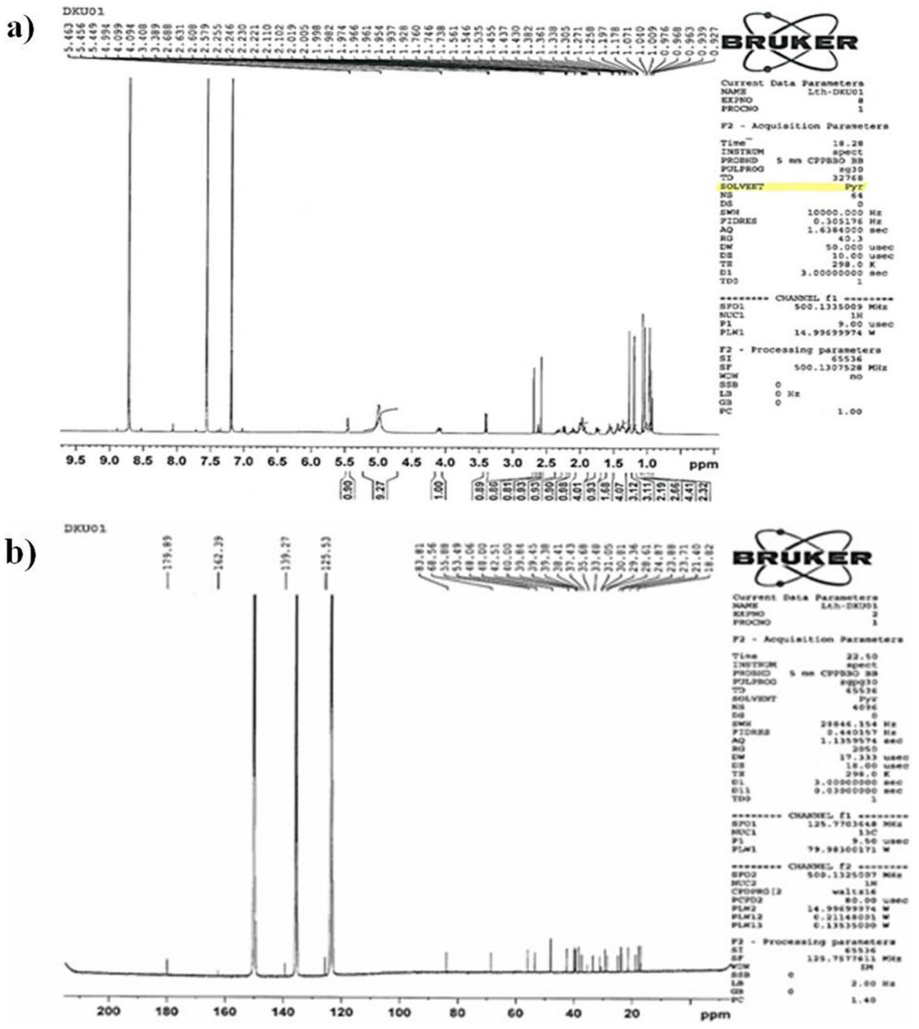


**Fig. 3.** Analysis of chloroform fraction for polysaccharide-removed extract after 70% ethanol extraction using NMR system. (a) $^{1}$H-NMR(pyridined5, 500 MHz)δ: 0.97(3H, d, J=6.5 Hz), 1.01(3H, d, J=6.5 Hz), 0.94, 1.03, 1.06, 1.20,1.25 (3H×5, s), 2.61(1H, d, J=11.0 Hz, H-18), 3.38(1H, d, J=9.5 Hz, H-3β), 4.08 (1H, td, J=11.0, 4.5 Hz, H-2β), 5.46 (1H, t-like, J=3.5 Hz, H-12). (b) $^{13}$C-NMRδ: 48.1 (C-1), 68.7 (C-2), 83.9 (C-3), 40.1 (C-4), 55.8 (C-5), 18.9 (C-6), 33.7(C-7), 40.2(C-8), 47.8(C-9), 37.3 (C-10), 24.1(C-11), 128.19(C-12), 140.1(C-13), 42.4(C-14), 29.5(C-15), 26.3 (C-16), 48.3(C-17), 54.4 (C- 18), 72.4(C-19), 42.2 (C-20), 27.1(C-21), 38.5 (C-22), 29.6 (C-23), 22.2 (C-24), 16.8 (C-25), 17.5(C-26), 24.6 (C-27), 180.7(C-28), 27.3(C-29), 16.6 (C-30).

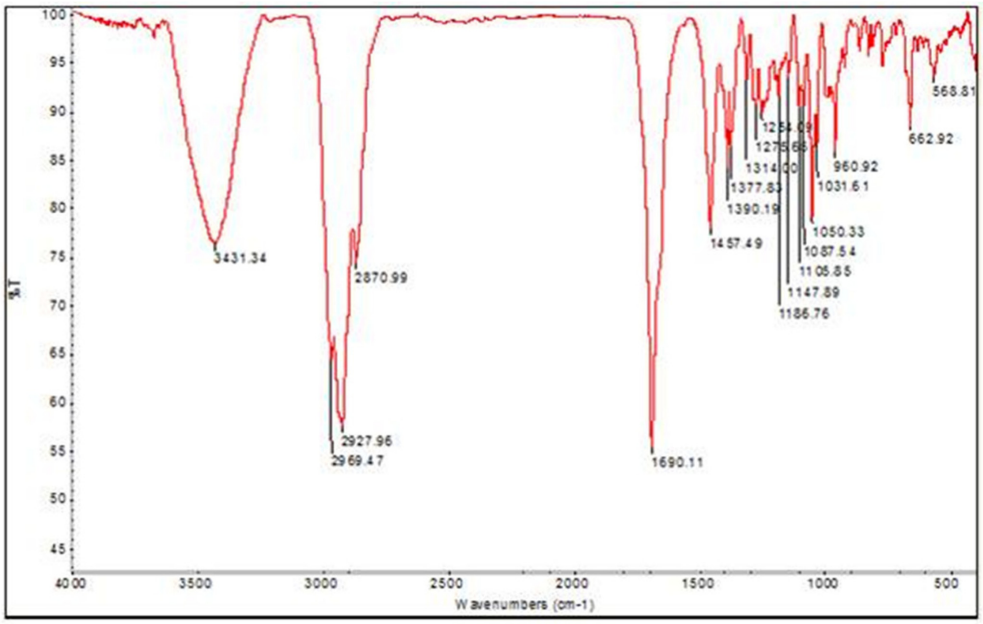


**Fig. 4.** Analysis of chloroform fraction for polysaccharide-removed extract after 70% ethanol extraction using IR system.

the highest downregulating effect on MMP-1 and MMP-2 a (Fig. 2).

To determine the active compound responsible for the anti-wrinkle efficacy of the fraction, the chloroform fraction was analyzed using HPLC, NMR, IR, EA, and MS. The $^{1}$H-NMR spectra show cyclohexane peaks (0.97 ppm, 1.01 ppm, 0.94 ppm, 1.03 ppm, 1.06 ppm, 1.20 ppm, 1.25 ppm, 2.61 ppm) and specific –OH peak (3.38 ppm). The$^{13}$C-NMR spectra show C=O peak (180.7 ppm) and C=C peak (128.19 ppm) (Fig. 3). Cyclohexane, –OH, C=O, and C=C peaks were detected in DEPT-NMR and two-dimensional NMR spectra (data not shown). Data obtained from EA, IR, MS analyses reinforced that obtained from NMR analysis. In IR analysis, –COOH, –OH, and C=C peaks were detected confirming the presence of 30 carbons in the structure (Fig. 4). From the elemental analysis, the expected molecular formula of active compound was found to be $C_{30}H_{48}O_4$ and the molecular weight was 472 (data not shown). From the analyzed data, the active compound was verified as corosolic acid after comparison with the structure in chemical data library from SciFinder. HPLC confirmed the presence of corosolic acid as well (data not shown).

The antioxidant efficacy of banana leaf extract was examined

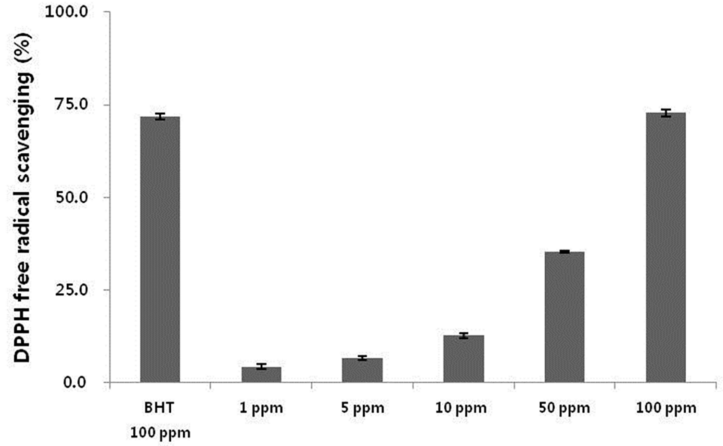


**Fig. 5.** Effect of banana leaves extracts on the DPPH free radical scavenging. The BHT (100 ppm) was used as positive control for the DPP free radical scavenging.

**Table 2.** Primers for genes investigated using RT-PCR analysis

| Gene | | Primer sequences |
|---|---|---|
| COL1A1 | F | 3' AGC CAG CAG ATC GAG AAC AT 5' |
| | R | 3' TCT TGT CCT TGG GGT TCT TG 5' |
| MMP-1 | F | 3' GAT GTG GAG TGC CTG ATG TG 5' |
| | R | 3' TGC TTG ACC CTC AGA GAC CT 5' |
| MMP-2 | F | 3' GTG CTG AAG GAC ACA CAC TAA AGA AGA 5' |
| | R | 3' TTG CCA TCC TTC TCA AAG TTG TAG G 5' |
| GAPDH | F | 3' AAC GAA TTT GGT CGA ACA GC 5' |
| | R | 3' TGA GGA GGG ATT CAG TG 5' |

* F: Forward, R: Reverse.

**Table 3.** The change of ΔR5 value

| | A (Test sample) | | B (Positive control) | | ΔR5 (8 weeks-before) | |
|---|---|---|---|---|---|---|
| | Before | 8 weeks | Before | 8 weeks | A | B |
| Means | 15.46 | 13.87 | 14.36 | 13.15 | -1.59 | -1.21 |
| Standard deviation | 3.56 | 2.06 | 3.01 | 2.04 | 3.09 | 2.5 |
| *p*-value (A for B) | | | 0.499 | | | |

*Positive control: 0.04% Adenosine containing cream.
*$p<0.05$, **$p<0.01$, ***$p<0.001$: p-value is measured by paired *t*-test.

**Table 4.** The change of Δscore

| | A (Test sample) | | B (Positive control) | | Δscore (8 weeks-before) | |
|---|---|---|---|---|---|---|
| | Before | 8 weeks | Before | 8 weeks | A | B |
| Means | 2.77 | 2.31 | 2.69 | 2.27 | -0.46 | -0.42 |
| Standard deviation | 1.03 | 0.84 | 1.16 | 1.04 | 0.65 | 0.58 |
| *p*-value (A for B) | | | 0.814 | | | |

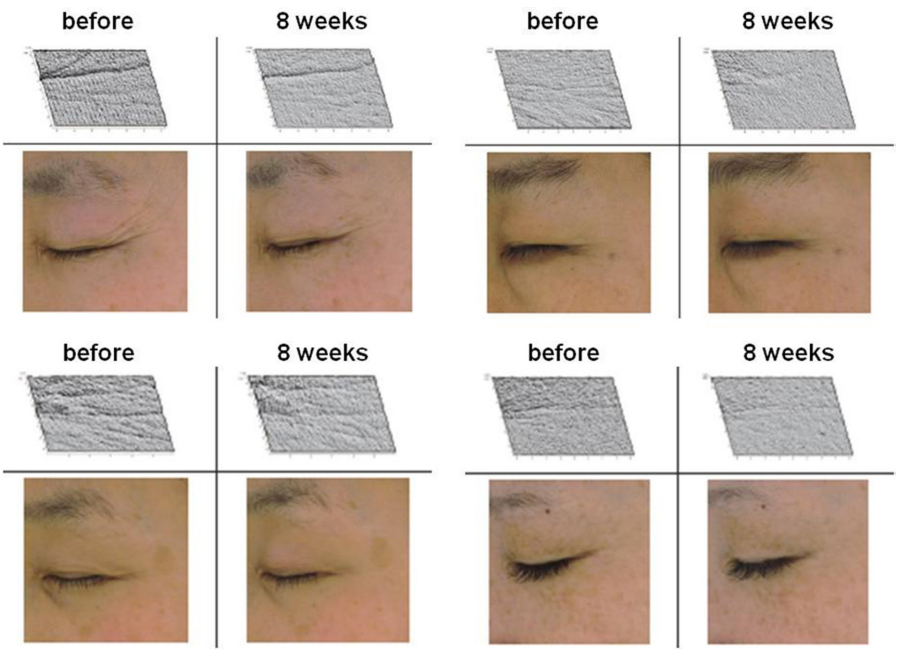


**Fig. 6.** Change of crow feet and the winkles that were applied with Banana leaves extract containing cream on volunteers for 8 weeks.

by DPPH free radical scavenging assay. As shown in Fig. 5, the banana leaf extract upregulated DPPH free radical scavenging in a dose-dependent manner. The extract showed antioxidation efficacy with an $IC_{50}$ of 67.91 ppm, which was comparable to

that of the positive control; BHT (100 ppm).

Banana leaf extract containing-cream was applied on volunteers' crow's feet and the winkles were analyzed. Post cream application for 8 weeks, the average R1, R2, R3, R4, and R5 were -10.32, -9.73, -4.86, -7.60, and -1.59, respectively (Table 2). The antiwrinkle efficacy of the extract was similar to that of the positive control, cream-containing adenosine, a functional wrinkle care ingredient in Korea. Dermatologic evaluation of the difference in wrinkle level from initiation of clinical test (score) to 8 weeks after applying the banana leaf extract-containing cream was -0.46 (Table 3). As shown in Fig. 6, crow's feet and winkles clearly decreased in volunteers who applied banana leaf extract-containing cream for 8 weeks. It was clear from these results that banana leaf extract has good potential for use as a cosmetic raw material in aiding wrinkle care.

## 4. CONCLUSION

Many plant extracts have been examined for the presence of potential antiaging ingredients. For example, green tea [12], blackberry [13], *Kaempferia pandurata* [14], and *Pothomorphe umbellata* [15] extracts have demonstrated inhibitory effects on aging activities.

In this study, we have shown that banana leaf extract was not only innocuous to human skin fibroblasts but also significantly decreased the expression of both *MMP-1* and *MMP-2* and increased the expression of *COL1A1*. Besides the antiwrinkle effect, banana leaf extract also showed a dose-dependent scavenging activity against DPPH free radicals. By using several analysis, we have concluded that the active ingredient of the extract is corosolic acid. The antiwrinkle effect of corosolic acid has been reported; corosolic acid, and consequently, the anti-wrinkle effect of the banana leaf extract could be attributed to corosilic acid. Furthermore, we confirmed the safety and antiwrinkle effect on human skin through clinical testing.

Based on the results observed in this study, *Musa sapientum* L. (Banana) leaves, which are the unused portion of banana, could be utilized as an excellent antiaging ingredient.

## REFERENCES


1. Seo, J. Y., E. K. Kim, S. H. Lee, K. C. Park, K. H. Kim, H. C. Eun, and J. H. Chung (2003) Enhanced expression of cylooxygenase-2 by UV in aged human skin in vivo. *Mech Ageing Dev.* 124: 903.
2. Nichols, J. A. and S. K. Katiyar (2009) Skin photoprotection by natural polyphenols: anti-inflammatory, antioxidant and DNA repair mechanisms. *Arch. Dermatol. Res.* 302: 71.
3. Hashem, M. A., K. Y. Jun, E. Lee, S. Lim, H. Y. Choo, and Y. Kwon (2008) A rapid and sensitive screening system for human type I collagen with the aim of discovering potent anti-aging or anti- fibrotic compounds. *Mol. Cells* 26: 625-630.
4. GA. Lullo. D., Sweeney. S. M., Korkko. J., Ala-Kokko. L., and San. A. JD (2002) Mapping the ligand-binding sites and disease associated mutations on the most abundant protein in the human, type I collagen, *J. Biol. Chem*. 277: 4223-4231.
5. Jurkiewicz, B. A., D. L. Bissett, and G. R. Buttner (1995) Effect of topically applied tocopherol on ultraviolet radiation-mediated free radical damage in skin. *J. Invest. Dermatol*. 104: 484-488.
6. Dai, G., Freudenberger, T., Zipper, P., Melchior, A., Grether-Beck, S., Rabausch, B., de Groot, J., Twarock, S., Hanenberg, H., Homey, B., Krutmann, J., Reifenberger, J., and Fischer, J. W. (2007) Chronic ultraviolet B irradiation causes loss of hyaluronic acid from mouse dermis because of down-regulation of hyaluronic acid synthases. *Am. J. Pathol.* 171: 1451-1461.
7. Jenkins, G. (2002) Molecular mechanisms of skin ageing. *Mech. Ageing Dev.* 123: 801-810.
8. Werb, Z. (1997) ECM and cell surface proteolysis: regulating cellular ecology. *Cell* 14: 439-442.
9. Kahari, V. M. and U. Saarialho-Kere (1997) Matrix metalloproteinases in skin. *Exp. Dermatol.* 6: 199-213.
10. Curran, S. and G. I. Murray (1999) Matrix metalloproteinases in tumor invasion and metastasis. *J. Pathol.* 189: 300-308.
11. Sternlicht, M. D. and Z. Werb (2001) How matrix metalloproteinase regulate cell behavior. *Ann. Rev. Cell Dev. Bi* 17: 463-516.
12. Katiyar, S. K. and C. A. Elmets (2001) Green tea polyphenolic antioxidants and skin photoprotection. *Int. J. Oncol.* 18: 1307-1313.
13. Herrmann, M. S. Grether-Beck, I. Meyer, H. Franke, H. Joppe, J. Krutmann, and G. Vielhaber (2007) Blackberry leaf extract: a multifunctional anti-aging active. *Int. J. Cosmet. Sci.* 29: 411.
14. Shim, J. S., E. J. Choi, C. W. Lee, H. S. Kim, and J. K. Hwang, (2009) Matrix metalloproteinase-1 inhibitory activity of Kaempferia pandurata Roxb. *J. Med. Food* 12: 601-607.
15. Ropke, C. D., V. V. da Silva, C. Z. Kera, D. V. Miranda, R. L. de Almeida, T. C. Sawada, and S. B. Barros (2006) In vitro and in vivo inhibition of skin matrix metalloproteinases by Pothomorphe umbellata root extract. *Photochem. Photobiol.* 82: 439-442.
16. Zong, W. and G. Zhao (2007) Corosolic acid isolation from the leaves of *Eriobotrta japonica* showing the effects on carbohydrate metabolism and differentiation of 3T3-L1 adipocytes. *Asia Pac. J. Clin. Nutr.* 16: 346-352.
17. Kim, M. S., Y. K. Kim, K. H. Cho, and J. H. Chung (2006) Regulation of type I procollagen and MMP-1 expression after single or repeated exposure to infrared radiation in human skin. *Mech. Ageing Dev.* 127: 875-882.
18. Steinbrenner, H., M. C. Ramos, D. Stuhlmann, H. Sies, and P. Brenneisen (2003) UVA-mediated downregulation of MMP-2 and MMP-9 in human epidermal keratinocytes. *Biochem. Biophys. Res. Co.* 308: 486-491.
19. Mildner, M., W. Weninger, F. Trautinger, J. Ban, and E. Tschachler (1999) UVA and UVB radiation differentiallyregulate vascular endothelial growth factor expression in keratinocyte-derived cell lines and in human keratinocytes. *Photochem. Photobiol.* 70: 674-679.

20. Pearse, A. D., S. A. Gaskell, and R. Marks (1987) Epidermal changes in human skin following irradiation with either UVB or UVA. *J. Invest. Dermatol*. 88: 83-87.
21. Beissert. S. and R. D. Granstein (1996) UV-induced cutaneous photobiology. *Crit. Rev. Biochem. Mol. Biol*. 3: 381-404.
22. Fisher, G. J., Z. Q. Wang, S. C. Datta, J. Varani, S. Kang, and J. J. Voorhees (1997) Pathophysiology of premature skin aging induced by ultraviolet light. *N. Engl. J. Med.* 337: 1419-1428.
23. Talwar, J. S., C. E. M. Griffiths, G. J. Fisher, T. A. Hamilton, and J. J. Vorhees (1995) Reduced type I and type III procollagens in photodamaged adult human skin. *J. Invest. Dermatol.* 105: 285-290.
24. Fisher, G. J., S. Kang, J. Varani, Z. Bata-Csorgo, Y. Wan, S. Datta, and J. J. Bpprjees (2002) Mechanisms of photoaging and chronological skin ageing. *Arch. Dermatol.* 138: 1462-1470.
25. Pinnell, S. R., S. Murad, and D. Darr (1987) Induction of collagen synthesis by ascorbic acid. A possible mechanism. *Arch. Dermatol.* 123: 1684-1686.