POTENTIAL ANTI-WRINKLE EFFECTS OF M. SPAIENTUM L. LEAVES EXTRACT

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ABSTRACT
To verify the efficacy of M. spainentum L. leaves extract as a cosmetic raw material, active compound of Banana leaves extract and anti-wrinkle efficacy was determined.

METHODS
a) Preparation of M. spainentum L. leaves extract:
Dried Banana leaves were extracted with water (80 degree Celsius) or 70% ethanol (60 degree Celsius) for 4 hours. Crude extracts were concentrated with filtration, re-crystallization and drying. Purified extract were obtained from dried crude extract with removal of polysaccharide.

b) Cell culture
HS68 cells were cultivated in DMEM with 10% fetal bovine serum and 100 U/ml penicillin/streptomycin at 37°C in a humidified atmosphere with 5% CO2.

c) Cell viability assay
Cell viability was assessed by conventional MTT assays. The absorbance of the samples was measured at 540 nm with a microplate reader.

d) Enzyme-linked immunosorbent assay
To determine procollagen expression levels, PIP EIA Kit was used, according to the manufacturer’s instructions.

e) mRNA analysis by semi-quantitative RT-PCR
To determine mRNA expression levels, total RNA was isolated from cells with TRIzol Reagent, according to the manufacturer’s instructions. Analysis of mRNA was also performed using semi-quantitative RT-PCR. The results were expressed as the ratio of optical density 280 nm to GAPDH mRNA concentration.

f) Analysis
Analysis of active ingredient from Banana leaves extract was performed using HPLC, NMR, IR, EA and MS.

RESULTS
Purified extract from 70% ethanol extract show most effective for anti-wrinkle efficacy within prepared samples. The active compound of banana leaves extract was analyzed with HPLC, NMR, IR, EA and MS identified to corosolic acid.

Further studies such as in vivo efficacy test, stability, safety, delivery and etc will be conducted for developing new cosmetic raw material using banana leaves.

Key words: M. spainentum L., Banana leaf, Anti-wrinkle, Anti-aging, Cosmetics

INTRODUCTION
Aging is atrophied changes that are degraded the functions of physical and psychological with age. In general, skin aging appeared by many different kinds of symptoms; xeroderma, skin tone, the change of skin elasticity, vasodilation and wrinkles[1, 2, 3]. From among these symptoms, wrinkles are classified between intrinsic aging and photo-aging. Wrinkles are influenced by age, external environment and UV-irradiation. Skin damages by these factors cause many kinds of the aging process; increase of skin stress, stratum corneum hypertrophy, decrease of stratum corneum moisture and decrease of elasticity

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and flexibility [4]. The mechanism of photo-aging is known that decrease of synthesis the collagen and over expression of MMPs (matrix metalloproteinases) by UV-irradiation, so cause many kinds of the aging process like wrinkles [5, 6]. MMPs are enzymes that are directly responsible for the degradation of ECM (extracellular matrix) components such as collagen, gelatin, and elastin. MMP-1, an interstitial collag enase, 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 ECM components, such as collagen, by increasing the production of MMPs in the skin. Therefore, the regulation of MMP activities may protect the skin from UV-induced damage. This is the rationale for most bioassays searching for agents able to protect the skin from UV-induced overexpression of MMPs.

Polysaccharides are very common in nature serving as plant building material (cellulose). Polysaccharides also serve highly special functions such as protective coatings for bacteria and other cells. However, it is hard to use as cosmetic ingredient because of dark color. Therefore, we performed the polysaccharide removal process in this study. Polysaccharide remove may cause reduction of dark color and specific odor. Polysaccharide remove can also increase the skin absorption rates.

M. spiantum L. (Banana) is mainly grown in the tropical and subtropical countries and is widely used for its nutritional values all over the world. The fruits of the plant are used to treat different diseases in human in traditional medicine; diarrhoea, dysentery, intestinal lesions in ulcerative colitis, diabetes, uremia, nephritis, gout, hypertension and cardiac disease [7]. But these usages were confined to the fruits, so most other parts are discarded. This is very inefficient in industrial and environmental point. If we study new ways of using the discarded parts, it will bring positive effects not only the possibility through new materials but also environmental aspects.

**MAIN BODY**

a) **M. spiantum L. leaves**

*M. spiantum L.* (Banana) leaves were purchased from banana farm in Jeju Island, Korea where certified as an eco-friendly certification. Purchased banana leaf were cleaned with tap water and dried. Dried leaves were kept in dried container until use.

b) **Extraction**

Banana leaves were extracted as following scheme 1.

c) **Effect of Banana leaves extracts on the production of wrinkle mediators.**

Extrinsic and intrinsic skin aging commonly increases wrinkling, sagging, and laxity [8]. Extrinsic aging is generally related to photoaging 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 [9], dermal photoaging [10], and several pathologies such as tumorigenesis [11]. 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 the elevated activities of other MMPs [12].

The expression of COL1A1, MMP-1, and MMP-2 genes and procollagen involved in photoaging and the subsequent formation of wrinkles were examined by RT-PCR and ELISA in human fibroblasts treated with the *Banana* leaves extracts. As shown in Figure 1, banana leaves extract regulated wrinkle-related gene expression. Especially, the polysaccharide-removed 70% ethanol extract has shown the greatest up-regulating effect on procollagen and COL1A1, and the greatest down-regulating effect on MMP-1 and MMP-2. As a result, polysaccharide removed banana leaf extract has excellent anti-wrinkle efficacy.

d) **Effect of solvent for extraction on anti-wrinkle efficacy**

To select the extraction solvent, banana leaves were extracted with solvents such as hexane, chloroform, ethyl acetate, methanol, ethanol and water respectively and compared the gene expression of COL1A1, MMP-1, and MMP-2 with each extract. Banana leaves extract using chloroform show most up-regulating effect on procollagen and COL1A1, and the most down-regulating effect on MMP-1 and MMP-2 in a dose-dependent manner (Fig. 2).

e) **Identifying the active compound from banana leaves.**

To find the active compound that shows the anti-wrinkle efficacy, banana extract were analyzed with HPLC, NMR, IR, EA and MS. From the result of $^1$H-NMR Spectrum, the 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) were detected. On the $^1$C-NMR Spectrum, the C=O peak (180.7 ppm) and C=C peak (128.19 ppm) were detected (Fig. 4). EA, IR, MS analyzed data also fortify the NMR analyzed data. The expected molecular formula of active compound is $C_{28}H_{34}O_4$ and the molecular weight is calculated with 472 (Fig 5). From these analyzed data the active compound is verified as corosolic acid and the result also compared with chemical data library from scifinder (Fig 6). Using HPLC, detection of corosolic acid was confirmed again (data not shown).
FIGURES AND TABLES

Scheme: 1. Extraction of banana leaves.

Figure: 1. Effect of *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) Effect of *Banana* leaves extracts on the expression of wrinkle-mediated genes. Levels of mRNAs were determined by semi quantitative RT-PCR.

(*HWE: hot water extract, EE: Ethanol extract, PR: polysaccharide-removed)
Figure: 2. Effect of each fraction for polysaccharide-removed extract after 70% ethanol extraction 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 these samples for 24 h. b) Effect of these samples on the expression of wrinkle-mediated genes. Levels of mRNAs were determined by semi quantitative RT-PCR.
Figure 3. Analysis of chloroform fraction for polysaccharide-removed extract after 70% ethanol extraction using NMR system.

a) $^1$H-NMR(pyridined5, 500MHz)$\delta$: 0.97(3H,d,J=6.5Hz), 1.01(3H, d, J=6.5Hz), 0.94, 1.03, 1.06, 1.20, 1.25 (3H×5, s), 2.61(1H, d, J=11.0Hz, H-18), 3.38(1H, d, J=9.5Hz, H-3β), 4.08 (1H,td, J=11.0, 4.5Hz, H-2β) , 5.46 (1H, t-like, J=3.5Hz, H-12).

b) $^{13}$C-NMR$\delta$: 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).

Figure 5 Structure of corosolic acid.

CONCLUSION

Many plant extracts have been examined for the presence of potential MMP inhibitors. For example, green tea [13], blackberry [14] and Kaempferia pandurata [15] have demonstrated inhibitory effects on MMPs activities. In this study, we have shown that Banana leaves extract has the excellent efficacy for the anti-wrinkle effect. Furthermore, by using several analysis, we have concluded that the active ingredient is corosolic acid.

Stand on the results observed in this study, it is necessary that further studies in vivo and in clinical tests by using dosage form, such as liposome containing Banana leaves extracts, by so doing, Banana could apply to excellent anti-aging ingredients, and by utilizing the leaves; unused portion of Banana, it is expected that impact on the promotion of farm income.

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REFERENCES


