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"Development of well aging skincare materials from insight of historical plant exudates"

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1. Introduction

According to a report from Great Britain's Royal Society for Public Health, mature women are rejecting the expression "anti-aging" and tend to have wellbeing, healthier lifestyle [1]. With the trend, the concept of well aging is emerging as an alternative of anti-aging even in cosmetic field. Because that "well aging" concept includes physical and mental condition together, more research is required to characterize specifically in terms of skin. Plant exudates (Pexs) including resin, oleoresin, gum and balsam have been applied for centuries as medicinal treatment. Therapeutic efficacy of exudates was confirmed by authorities like Dioscorides, Galen and Ibn Sina [2]. Among their various reported efficacies, Pexs applied in cosmetics exhibit remarkable properties such as wound healing and anti-inflammatory effects.

Mastic gum, the resin of *Pistacia lentiscus*, has been applied to treat skin inflammation since ancient times. Not only a heritage of Greece but with ethnopharmacological power, mastic gum has been referred by distinguished physicians like Galen, Pliny, Dioscorides. Especially, Dioscorides who is a great physician in 1st century AD described mastic as a skincare agent. More than 120 compounds have been analyzed in the resin and several pharmacological properties such as anti-inflammatory, antioxidant, anti-diabetic and anti-ulcer have been studied. Based on European Medicines Agency (EMA) monograph issued in 2015, mastic gum was recognized as a traditional herbal medicinal product for the treatment of mild dyspeptic disorders, minor skin inflammations and in healing of minor wounds [3,4].

Boswellia serrata gum called frankincense, or olibanum is a plant exudate tapped from trunk of the *Boswellia serrata*, which is one of the ancient and most valued herbs in Ayurveda. A Sanskrit name of Boswellia, "*Gajabhakshya*", suggests that elephants consumed this as a part of their diet. Being a significant source of Boswellic acid (BA) and several kinds of terpenes, the beneficial use of resin for skin and blood diseases, ringworm, boils, hair-loss etc. was mentioned in traditional Ayurvedic and Unani texts [5,6]. BAs are pentacyclic triterpenes with powerful anti-inflammatory activity and the effect of BAs on photo and age-damaged skin was identified via topical treatment [7].

Myrrh is an aromatic resin extracted from the *Commiphora* tree of which the cost was higher than gold. The Arabic term "Murr" means "Bitter", which describes the aroma of myrrh. Traditionally, the resin of *Commiphora myrrha* was applied to treat skin inflammation, ulcers, sinusitis in Britain and small wounds, infection of the buccal cavity in France. Also, it is used as

traditional medical practices in China, the Middle East, and Africa. Various phytochemicals, including terpenoids and polyphenols, were analyzed, and their antibacterial, antioxidant, and anti-inflammatory properties were investigated [5,8,9].

Recently, from skin protection to retinol degrading enzyme inhibition, research on variable Pexs is being subdivided into several dermal molecular mechanisms. Nevertheless, there is a limitation of the use in cosmetic field mainly because of poor-aqueous solubility and sensual stickiness. Based on previous studies, we confirmed characteristics and dermal activities of various kinds of Pexs especially *Boswellia serrata* gum, *Boswellia carterii* resin, *Commiphora myrrha* resin and *Pistacia lentiscus* (Mastic) gum extracted with respective extraction process regarding polarity. In addition, an exclusive Pexs concoction inspired by medieval beauty prescriptions was developed to maximize the efficacy for skin and formulation compatibility. In consideration of solubility and active compound extraction efficiency, 3 kinds of Pexs and some medicinal herbs were extracted via proper solvents including alcohol and polyol.

With skin well aging concept, reaction effectiveness from the viewpoint of age related dermal molecular change was investigated via in vitro experiments. As an example, it was confirmed that Pexs concoction has the effect of replenishing molecules involved in skin elasticity that decrease with age. Also, protection from skin cellular aging of 3 Pexs were studied via induced aging model.

Focused on maintaining heathier skin, the possibility of Pexs for skin well aging was verified. However, for the deeper understanding of Pexs concoction activity, further studies related to detailed molecular mechanisms of action and clinical efficacy are required.

2. Materials and Methods

2.1. Preparation of 3 Pexs and Pex concoction

P. lentiscus (Mastic) gum, *B. serrata* gum, *C. myrrha* resin were extracted with a solvent including wine, water and polyol at a proper rate. Nontoxic carbohydrate derivative, which is used to enhance solubility of the materials in aqueous solution was used as a solvent for increasing applicability of plant exudates. Each of 3 Pexs were treated after dilution with distilled water.

2.2. Cell culture and cytotoxicity

Hs68 fibroblast cells (Hs68 cells) obtained from ATCC were used for in vitro experiments. Cells were cultured with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and 1% penicillin–streptomycin and maintained at 37℃ in a humidified atmosphere with 5% CO_2. For 3 Pexs, cytotoxicity tests were performed. Hs68 cells (1.5×10^4 cells/well) were plated in 48-well plates for 24h. After, Different concentrations of 3 Pexs were treated for additional 24h. CCK assay kit (Dojindo) were applied according to kit manual and absorbance of each well at 450 nm was measured using microplate spectrophotometer.

2.3. Real Time PCR

For real-time PCR analysis, three individual Pexs and the Pex concoction were applied for 24 h after the cells reached approximately 90% confluency. RNA extraction and cDNA synthesis were performed with proper kits (Qiagen and AccuPower® RocketScript™ Cycle RT PreMix) according to kit manual. The cDNA samples were analyzed for each genes using TaqMan™ probes in QuantStudio 5 Real-Time PCR System (Applied Biosystems™). The cycling

conditions were as follows: initial hold for 2 min at 50℃, 10 min at 95℃, then 40 cycles with 15 sec at 95℃, 1 min at 60℃. Quantitation of gene expression was carried out by the comparative Ct method and the expression levels of target mRNAs were normalized by the GAPDH expression.

2.4 Cellular Senescence Induction and Chemical Compound Treatment

Hs68 cells (2×10^5 cells/well, population doubling level 19) were seeded in 6-well plated and maintained for 24h with 10% FBS including media. 2 kinds of induced cellular aging models were applied for this experiment. First, the H_2O_2 induced model was established modifying WEN 's study [10]. Several concentrations of H_2O_2 were tested to determine the final treatment concentration. Cells were first treated with 1% of each Pex for 24 h, followed by exposure to 300 μM H_2O_2 in serum-free (sf) media for 3 h. Subsequently, treatment with 1% Pexs in sf media was performed for an additional 24 h. A second model was built up with modification of An's study [11]. Cells were induced to senescence by treatment with 50 ng/mL doxorubicin plus 50 ng/mL IGF1 for 7d. Then, 1% of 3 Pexs with 50 ng/mL doxorubicin plus 50 ng/mL IGF1 were treated further 7d. All chemicals were renewed every 48h.

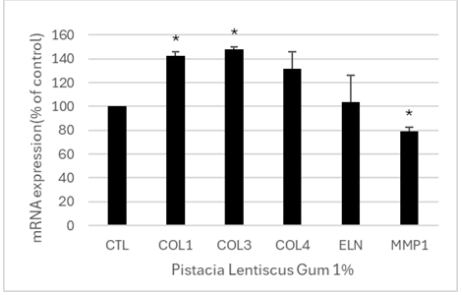
2.5. SA-β-Gal Activity Assay

Senescence β-Galactosidase Staining Kit (Cell signaling technology) was adapted to stain senescent cells. According to manufacturer's instructions, cells were washed with phosphate buffered saline (PBS) and fixed at room temperature for 10 minutes. Then, overnight incubation with staining working solution at 37℃ was conducted.

3. Results

3.1. Efficacy of 3 Pexs related to skin elasticity

No cytotoxicity effect of 3 Pexs were found (data not shown). The dermal layer gives skin its strength and elasticity due to the high contents of collagen and elastin fibers. However, these proteins decrease significantly with cutaneous ageing. To confirm efficacy of 3 Pexs on skin elasticity, different types of collagens, elastin and matrix metalloproteinase 1 (MMP1) were targeted. Type I collagen (COL1) is the predominant form in the dermis and gives the skin its elasticity and resilience. Type III collagen (COL3), which is abundant in fetal skin, plays a key role in maintaining homeostasis and promoting wound healing. Type IV collagen (COL4) is a net-forming collagen that attaches to each other to form complex protein networks. Elastin (ELN) is one of the important proteins which constitute elastic fiber in dermis. On the contrary, it is observed that MMP1, which is an initiating factor for collagen degradation, increases with age. A common effect observed across all three Pexs is the upregulation of COL3 expression and the suppression of MMP1 expression (Figure 1) [12–15].



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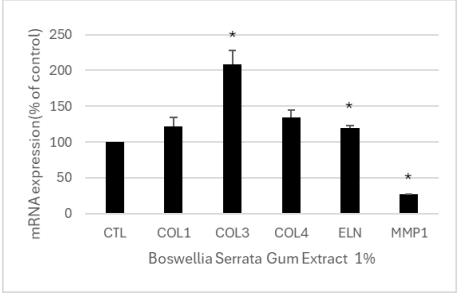

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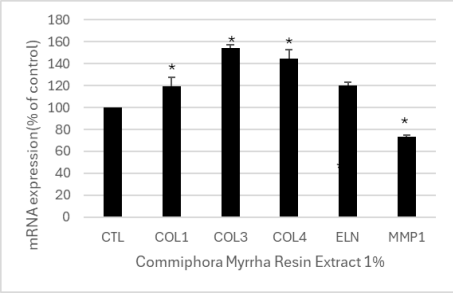

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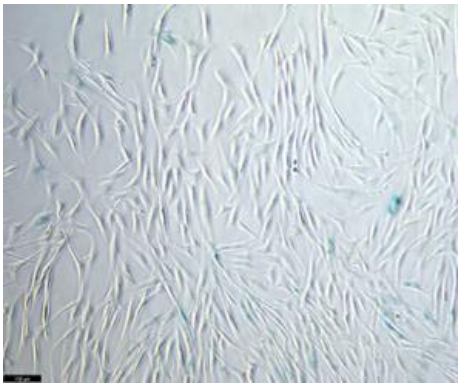
Figure 1. Dermal anti-aging effect of 3 Pexs by elastic fiber regulation**.** mRNA levels were determined by real-time PCR and normalized relative to that of GAPDH. (a) *P. lentiscus* gum revealed COL1, COL3 upregulation and MMP1 downregulation; (b) *B. serrata* gum extract increased COL3, ELN expression and decreased MMP1; (c) *C. myrrha* resin extract upregulated COL1, COL3, COL4 and downregulated MMP1 *P<0.05 versus control (CTL).

3.2. Positive effect of 3 Pexs on induced cellular senescence

Hydrogen peroxide (H_2O_2) is a representative chemical which induces oxidative stress that may contribute to dermal dysfunction. Activation of several kinases related to cellular senescence by H_2O_2 have been shown in previous studies. Owing to these properties, H_2O_2 has been applied to build an induced cellular senescence model. H_2O_2 induced cellular senescence model with a slight modification of WEN's study, pretreatment and additional treatment of 3 Pexs revealed possibility on cellular senescence regulation (Figure 2). In detail, SA-β-Gal positive cells were rarely found in control group, population doubling level 19. (Figure 2a) Relatively, H_2O_2 treatment group showed larger proportion of SA-β-Gal positive cells than control group that means H_2O_2 induced cellular senescence. (Figure 2b) However, 3 Pexs with H_2O_2 treatment groups showed reduced SA-β-Gal positive cell proportion compared to H_2O_2 treatment group, respectively. (Figure 2c-e)



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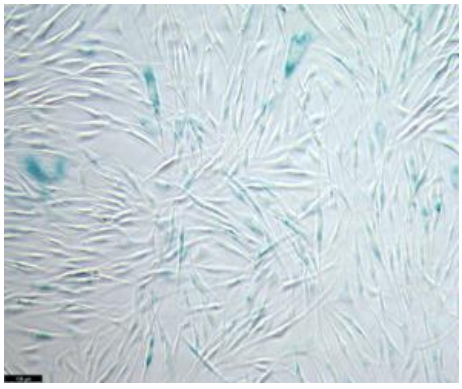

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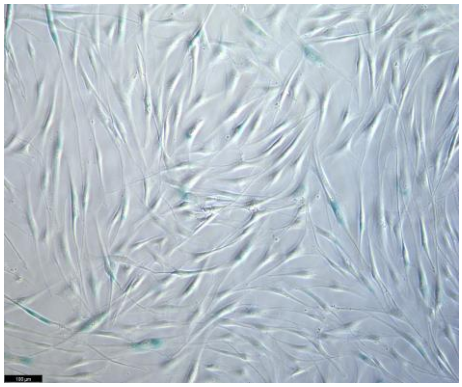

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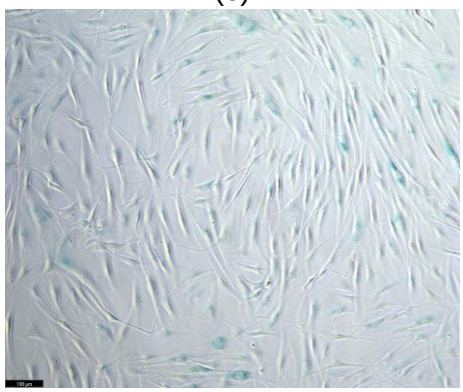

(**e**)

Figure 2. SA-β-gal staining was performed to identify the efficacy of 3 Pexs on H_2O_2 induced senescence. All Pexs were treated at a concentration of 1%. H_2O_2 induced senescence was certified with blue stained cells. 24h treatment of 3 Pexs were conducted before 3h of H_2O_2 treatment. After, 24h retreatment of 3 Pexs were carried out. 1% of *P. lentiscus* gum showed strong protection and recovery effect against aging. *B. serrata* gum and *C. myrrha* resin treatment cells showed possibility. (a) Control; (b) H_2O_2; (c) H_2O_2 with *P. lentiscus* gum; (d) H_2O_2 with *B. serrata* gum; (e) H_2O_2 with *C. myrrha* resin

Doxorubicin is a well-known chemical for DNA-damaging effects. According to An's study, the senescence condition was the proliferation condition plus the DNA-damaging chemical doxorubicin (100 ng/mL) via geroconversion. However, because DNA damage without growth stimuli is not sufficient to induce geroconversion, they added IGF1 and serum plus the DNA-damaging chemical doxorubicin (100 ng/mL) [11]. Because that 50 ng/ml treatment revealed better cell viability and similar SA-β-gal staining in our cell line, we identified the possibility of 3 Pexs on cellular senescence via modified treatment (Figure 3). Similary to H_2O_2 treatment group, doxorubicin and IGF-1 exposed group showed strong SA-β-Gal positive stain (Figure 3b) in comparison with control group (Figure 3a). In this induced senescence model, 3 Pexs were treated with doxorubicin and IGF-1 after 7 days exposure of doxorubicin and IGF-1. Another 7 days after, 1% of 3 Pexs treatment group (Figure 3c-e) showed relatively decreased proportion of SA-β-Gal positive cells as against to doxorubicin and IGF-1 treatment group.

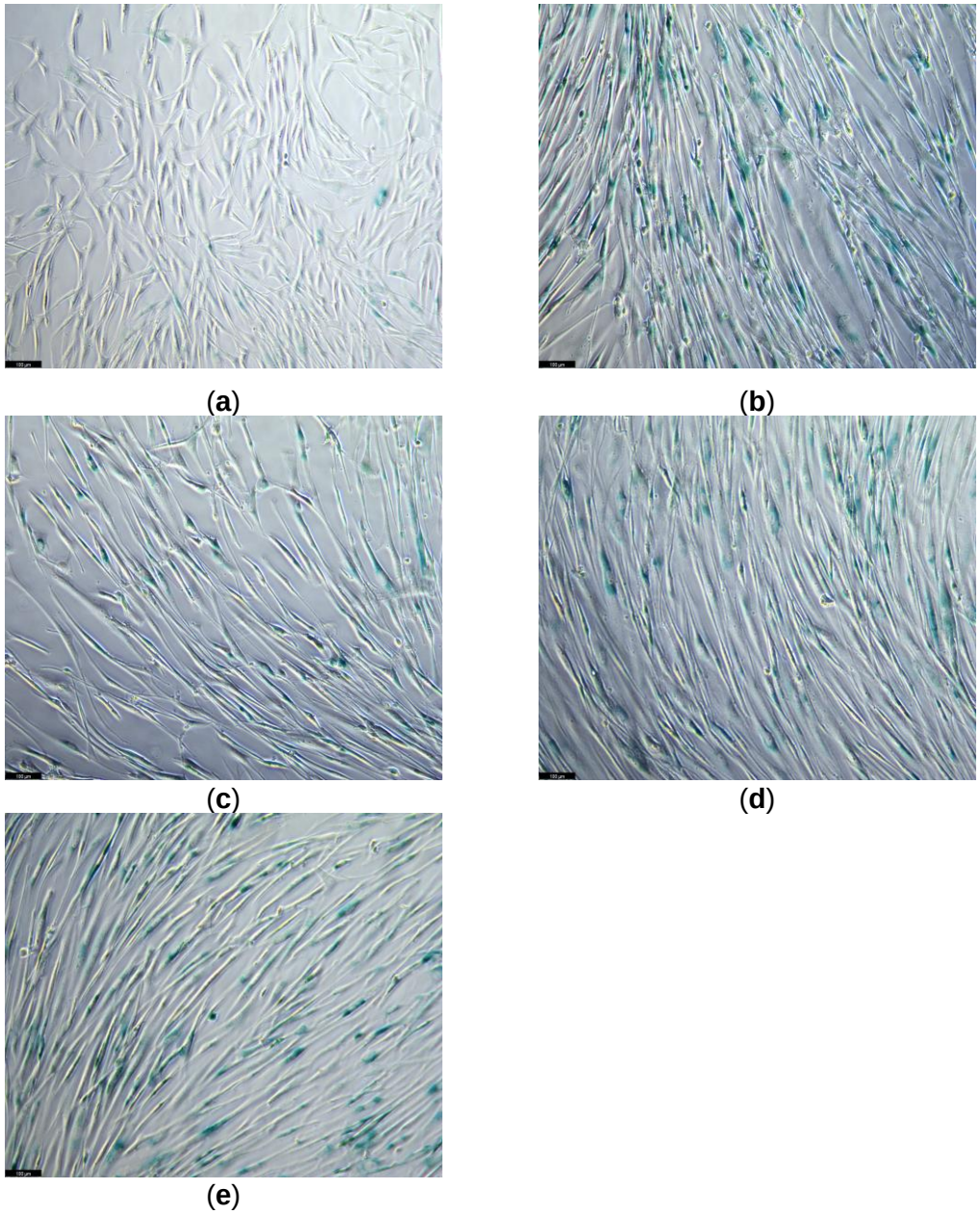

Figure 3. SA-β-gal staining was performed to identify the efficacy of 3 Pexs on doxorubicin plus IGF-1 induced senescence. All Pexs were treated at a concentration of 1%. (a) Control; (b) Doxorubicin + IGF-1; (c) Doxorubicin + IGF-1 with *P. lentiscus* gum; (d) Doxorubicin + IGF-1 with *B. serrata* gum; (e) Doxorubicin + IGF-1 with *C. myrrha* resin

4. Discussion

Plant exudates (Pexs) have been used since ancient times, due to their therapeutic and healing properties; in the perfume industry, in the cosmetics industry in fixative, preservative or aromatizer role. Normally, they are considered as healing treatments not only for the body, but also for mental health [16]. Basically, due to inflammatory, wound healing effect and aromatic properties of Pexs, applicability in the industry has always been discussed. However, because of chemical characteristics such as solubility, there was a limit to the actual efficacy confirmation in cosmetic fields. Among many kinds of Pexs, we focused on *P. lentiscus* gum, *B. serrata* gum and *C. myrrha* resin by referring to historical prescriptions. With polyol and carbohydrate

derivative, aqueous solubility of 3 Pexs was increased enough to apply for in vitro studies from a skin well-aging perspective.

The well-aging, a new concept of lifestyle, is emerging as a replacement for the existing concept, anti-aging. Aging, which is inseparable from living things, has been studied but has not been fully identified to these days. Especially, unlike the aging signs of internal organs, the skin demonstrates the obvious signs with the passage of time with the consequent impact on a person's social life. For this reason, anti-aging has been mainly promoted in the cosmetics industry. However, according to the Mintel's article, from 2004-2023, there has been a decline in searches for "anti-ageing" keywords on Google, while related to "healthy," "positive," and "well-ageing" have remained relatively stable. Nevertheless, how to evaluate dermal well-aging effect is still an uncertain situation. In this study, we focused on dermal molecular homeostasis and the power to respond to dermal cellular aging model for identifying skin well aging effect of 3 Pexs [17,18].

Based on essential factors related to skin aging signs exposed to the surface, the efficacy for sustaining homeostasis of 3 Pexs were evaluated in this study. The results demonstrated that 1% concentration of 3 Pexs increased several kinds of elastic fibers including diverse kinds of collagens, elastin respectively, decreased MMP1 of which breaks down collagen. Besides, 3 Pexs revealed efficacy for protection and recovery from 2 types of chemically induced cellular senescence models via SA-β-gal staining (Figure 2, 3).

5. Conclusion

The signs of skin aging can be easily identified through mirrors, and their findings can even affect the person's mind. As a result, it can have a negative impact on overall well-aging. Therefore, we considered responding to skin aging as the first step in overall well-aging and conducted a study on substances that exhibit this effectiveness. *P. lentiscus* gum, *B. serrata* gum and *C. myrrha* resin, being representative Pexs that contain ancient wisdom, have received attention and have gotten many attempts to apply. However, limitations in its practical application have been shown due to their characteristics. To overcome these limitations, Pexs extracted with specific solvents were utilized in this study. Being transformed into an appropriate formula for in vitro evaluation, the efficacy of Pexs for upregulation and downregulation related to dermal elastic homeostasis influenced by ageing has been identified. In addition, protection and recovery effect of 3 Pexs via H_2O_2 and doxorubicin induced cellular aging model respectively has been shown in this study. Despite the need for more detailed molecular mechanism studies and clinical investigation, all these data lead to potential of 3 Pexs for skin well-aging.

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