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Potential therapeutic properties of *Sorbus commixta* twig ethanol extract on vitiligo in skin cells

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Abstract: *Sorbus commixta* is a tree of the Rosaceae family growing in Asia that has long been used to treat asthma and neuralgia. In a previous report, the chemical isolated from the bark of *S. commixta* was shown to suppress the production of nitric oxide (NO) and preinflammation by downregulating the NF-κB pathway in lipopolysaccharide (LPS)-induced RAW 264.7 cells. Vitiligo is an acquired immune disease, usually characterized by white spots on the skin; however, its exact cause has not been identified. This study assessed the effects of an ethanol extract of *S. commixta* twigs (STE) on melanocyte activation, as well as its antiinflammatory and antioxidant properties. STE significantly increased the proliferation and melanin content of B16 melanocytes. Because of the importance of tumor necrosis factor (TNF)-α in inflammatory diseases, including the stimulation of vitiligo, the antiinflammatory effects of STE were tested in TNF-α-stimulated dermal fibroblasts and keratinocytes. STE reduced the levels of expression of IL-6, IL-8 and TNF-α mRNA and proteins. To assess the underlying molecular mechanism, the effects of STE on the mitogen-activated protein kinase (MAPK) signaling process were analyzed in dermal fibroblasts. Results show that STE inactivated extracellular signal-regulated kinase (ERK). In addition, STE exhibited antioxidative properties in assays of DPPH radical scavenging activity. Taken together, these findings suggest that STE has potential therapeutic activity in vitiligo.

Keywords: anti-inflammation; anti-oxidative effect; melanin synthesis; *Sorbus commixta* twig ethanol extract (STE); vitiligo

INTRODUCTION

Sorbus commixta is a small to medium-sized deciduous tree of the Rosaceae family, native to Korea, Japan and far east Russia, that grows on high, windy mountaintops and has long been used as a medicinal plant in East Asian countries. The cortex and bark of *S. commixta* have been used in Korean traditional medicine to treat various diseases, including asthma, bronchitis, gastritis and edema [1]. Vitiligo is the most common pigment disease worldwide, affecting about 0.5%-2% of the population and observed in both sexes and all races. Vitiligo is an acquired chronic depigmentation disorder of the skin resulting from the selective viability of melanocytes [2,3]. Multiple mechanisms are likely involved in melanocyte loss, including genetic predisposition, environmental triggers, oxidative stress, metabolic abnormalities, impaired renewal and altered immune responses [4]. Although the cause of vitiligo has not been determined, clinical studies over the past few years have suggested that vitiligo is largely an autoimmune condition [5].

Keratinocyte-derived factors can signal melanin production. For example, interleukin (IL)-6 secreted by keratinocytes can act on melanin-forming cells and suppress the expression of proteins associated with melanin production [6]. IL-6 and IL-8 can attract immune components to the skin, forming a link between triggering events and the initiation of autoimmune reactions that lead to the progression of vitiligo [7]. In addition, previous reports have shown an increase in tumor necrosis factor (TNF)-α associated with vitiligo. TNF-α can induce abnormal immune responses, which are frequently observed in patients with vitiligo [8-9]. TNF-α is a master cytokine that mediates inflammatory responses and innate immunity through nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and the mitogen-activated protein kinases (MAPK) signaling pathway [10].

Current understanding of the pathogenesis of vitiligo suggests that a successful treatment strategy should incorporate several distinct approaches, including the promotion of melanin production and melanocyte proliferation, and the regulation of immune responses [11]. Moreover, although water and ethanol extracts of *S. commixta* twigs showed anti-inflammatory and antioxidant properties [12], the pharmacological effects in models of vitiligo and its associated molecular mechanisms have not yet been fully investigated [1,12]. The present study investigates the pharmacological efficacy of STE in the treatment of vitiligo by analyzing its effects on melanin synthesis and melanocyte proliferation, as well as its anti-inflammatory and antioxidant properties in TNFα-stimulated dermal fibroblasts and keratinocytes.

MATERIALS AND METHODS

STE preparation

S. commixta twigs collected from Jiri-Mountain, Changwon-ri, Macheon-myeon, Hamyang-gun, Gyeongsangnam-do Province, South Korea were extracted in 80% ethanol for 48 h. The resultant STE was filtered, concentrated, and freeze dried to a powder within 72 h.

Cell culture

Melanocytes, fibroblasts and keratinocytes were used in this study. Mouse B16 melanoma cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA), and cultured in Dulbecco's modified Eagle's medium (DMEM; Welgene, Korea), containing 5% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) and a 1% penicillin-streptomycin mixture (Lonza, Basel, Switzerland), at 37°C in an atmosphere containing 5% CO_2. Primary normal human dermal fibroblasts (NHDFs; PromCell, Heidelberg, Germany) as dermal fibroblasts were used. NHDFs were cultured in fibroblast growth medium 2 (FGM-2; PromoCell, Germany). The human adult low calcium high temperature (HaCaT) keratinocyte cell line was used as dermal keratinocytes. HaCaT cells were kindly provided by the Amorepacific Corporation R&D Center, Shanghai, China, and grown in DMEM containing 10% FBS and 1% penicillin-streptomycin mixture.

Cell viability assay

Viability and cytotoxicity were evaluated using a cell counting kit-8 assay (CCK-8 DOJINDO, Tokyo, Japan). B16 cells were seeded onto 96-well plates (1.0×10^4 cells/well). Test samples were added at different concentrations, and the cells were incubated for 48 h. A 10-μL aliquot of CCK-8 solution was added to each well, and the plates were incubated for 1 h at 37°C. The absorbance of each well at 450 nm was measured using a Synergy™ HTX Multi-Mode Microplate Reader (Biotek, Winooski, VT, USA).

Measurement of melanin contents

B16 cells were seeded at a density of 2.0×10^4 cells/well in 48-well plates overnight and treated for 72 h with the indicated concentrations of STE. The cells were lysed with 1 N NaOH at 60°C for 30 min and absorbance was measured at 490 nm. The protein concentration of each sample was determined with the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA).

Sandwich enzyme-linked immunosorbent assay (ELISA)

HaCaT and NHDF cells (5×10^4 cells/well) were seeded onto 24-well plates, pretreated with STE for 3 h, and incubated with 10 ng/mL recombinant human TNF-α (R&D Systems, Minneapolis, MN, USA) for 10 min. The concentrations of IL-6 and IL-8 in the culture media were determined using ELISA Kits (R&D Systems), according to the manufacturer's instructions.

Real-time polymerase chain reaction (RT-PCR)

Total mRNA was prepared from HaCaT and NHDF cells using TRIzol reagent (Thermo Fisher Scientific), followed by reverse transcription using the GoScript™ Reverse Transcription System (Promega, Madison, WI, USA). Amplification was performed in 15-μL mixtures using iQ™ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA) and primers for human IL-6 (Bio-Rad,

BR186qHsaCID0020314-1000), human IL-8 (Bio-Rad, BR186qHsaCED0046633-1000), human TNF-α (BR186qHsaCED0037461-1000) and human glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Bio-Rad, BR186qHsaCED0038674-1000). The amplification protocol consisted of an initial denaturation at 95°C for 3 min, followed by 29 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 30 s and extension at 72°C for 30 s. All RT-PCR results were normalized relative to those for GAPDH in the same sample.

Western blotting

NHDF cells were seeded onto 6-well plates (15×10^4 cells/well) for 24 h, pretreated with 2.5-20 μg/mL STE for 3 h and stimulated with TNF-α (10 ng/mL) for 10 min. The cells were washed three times with ice-cold phosphate-buffered saline (PBS) and lysed in ice-cold RIPA buffer (Cell Signaling Technology, Danvers MA, USA) containing proteases and phosphatase inhibitors. The cell lysates were centrifuged at 10000 x *g* for 20 min at 4°C. Protein concentrations were quantified using bicinchoninic acid (BCA) Protein Assay Kits (Thermo Fisher Scientific, Waltham, MA, USA). Aliquots containing 30 μg of total protein were loaded onto 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels, electrophoresed and transferred to nitrocellulose membranes. The membranes were blocked with 5% skim milk in Tris-buffered saline (TBS) at room temperature for 1 h and incubated overnight at 4°C with primary antibodies against extracellular signal regulated kinase (ERK), phospho-ERK, c-Jun N-terminal kinase (JNK), phospho-JNK, p38, phospho-p38, NF-κB and β-actin (Cell Signaling Technology, Beverly, MA, USA). After three washes with Tris-buffered saline-0.1% Tween-20 (TBST), the membranes were incubated with secondary antibody at room temperature for 1 h. Bands were visualized using enhanced chemiluminescence solution (Bio-Rad), and images were processed using an iBright™ CL750 Imaging System (Invitrogen, Carlsbad, CA, USA).

Measurement of 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging ability

The radical scavenging ability of the antioxidant DPPH was measured as described. Briefly, 10 μL of extract solution was mixed with 190 μL of 0.1 mM DPPH solution, followed by incubation in the dark room for 10 min. Absorbance was measured at 517 nm with an ELISA reader.

Statistical analysis

Results are presented as the mean±standard deviation (SD) of three independent determinations. The statistical significance of between group differences were assessed using two-sided Student's t-tests, with P-values<0.05 considered statistically significant.

RESULTS

STE increased the proliferation and melanin contents of B16 cells

To determine its effects on the proliferation of B16 cells, cells were treated with different concentrations of STE for 48 h. Compared with control cells, STE at concentrations of 3.125-12.5 μg/mL increased cell proliferation (Fig. 1A), with 12.5 μg/mL enhancing cell proliferation to 136%. However, 50 μg/mL STE exhibited cytotoxicity.

STE also enhanced melanin synthesis, with 20 μg/mL STE increasing extracellular melanin to 186% and intracellular melanin to 220% (Fig. 1B). The color of the growth medium was darker in cells treated with STE. Moreover, analysis of melanocyte morphology and color showed that melanocytes treated with STE were darker than control melanocytes (Fig. 1C). α-melanocyte stimulating hormone (α-MSH) was used as a reference material for melanogenesis.

STE inhibited the production of cytokine mRNA and protein in NHDF and HaCaT

Fibroblasts and keratinocytes regulate the function of skin melanocytes. To test the antiinflammatory effects of STE, NHDF and HaCaT cells were treated with recombinant human TNF-α as a proinflammatory stimulus, and the mRNA levels of the representative proinflammatory cytokines IL-6, IL-8 and TNF-α were measured [13]. Because 200 μg/mL STE was cytotoxic to NHDF cells, cells were treated with 100 μg/mL STE, which stimulated cell proliferation (Fig. 2A). Pretreatment of NHDF cells with STE prior to stimulation with TNF-α

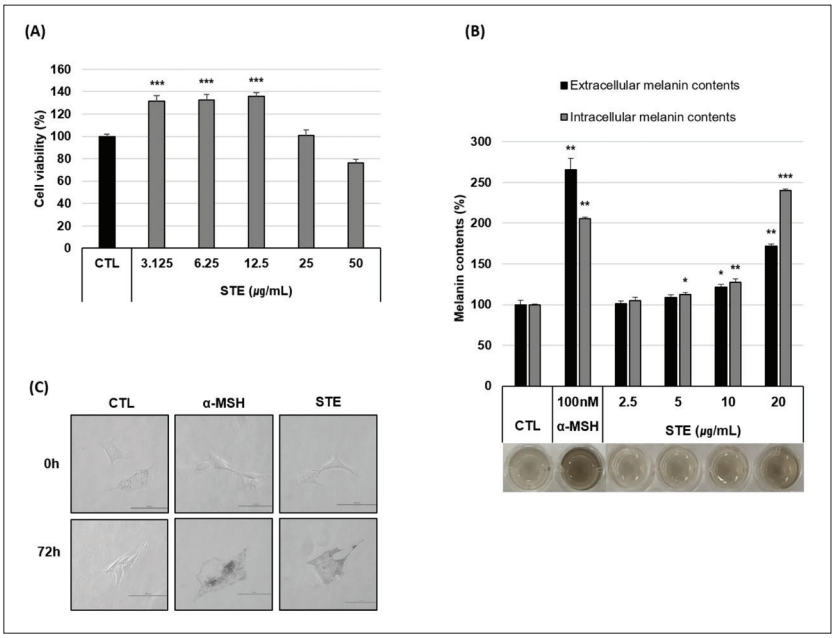

Fig. 1. Effect of STE on the proliferation and melanin content of B16 cells. B16 cells were incubated with STE for 48 h. **A** – Cell proliferation using CCK-8 assays; **B** – melanin content. To confirm the induction of melanin, cells were incubated with 100 nM alpha-melanocyte stimulating hormone (α-MSH) as a positive control, and the color of cell lysates and cultured media in 48-well plates was measured. **C** – Photographs taken with an optical microscope of B16 cells incubated with STE. B16 cells were seeded overnight onto 6-well plates at a density of 5.0×10^3 cells/well incubated for 72 h with 20 μg/mL STE or 100 nM with α-MSH as a positive control (scale bar 100 μm). The results are presented as the mean expression level obtained from three independent experiments. Data are presented as the mean±SD. *P<0.05, **P<0.01, ***P<0.001 compared with the control group.

Table 1. Effects of STE on IL-6 and IL-8 production in TNF-α-stimulated NHDF and HaCaT cells

Cells	Stimuli	STE Concentration (μg/mL)	Inhibition rate (%)	
			IL-6 (%)	IL-8 (%)
NHDF	Control (untreated)		100±0.67	100±0.37
	TNF-α (10 ng/mL)	0	0±1.38	0±6.89
		12.5	14.00±7.23 *	4.00±2.87
		25	17.00±4.33 **	8.00±5.15
		50	32.00±3.68 **	15.00±1.45 *
		100	40.00±3.89 ***	29.00±4.60 **
HaCaT	Control (untreated)		100±3.17	100±5.86
	TNF-α (10 ng/mL)	0	0±13.30	0±7.81
		3.125	8.00±2.67	20.00±12.82
		6.25	17.00±9.24	5.00±11.67
		12.5	23.00±3.17 *	24.00±1.24 **
		25	58.00±6.62 **	0±10.40

*P< 0.05; **P<0.01, ***P<0.001 compared with cells treated with TNF-α alone

dose-dependently decreased IL-6 mRNA levels as well as significantly reducing the levels of IL-8 and TNF-α mRNA (Fig. 2B). ELISA also showed that STE significantly reduced IL-6 and IL-8 protein production in NHDF cells (Fig. 2C and Table 1).

At a concentration of 50 μg/mL, STE displayed cytotoxicity in HaCaT cells (Fig. 2D). Pretreatment of these cells with STE dose-dependently reduced the expression of IL-6 mRNA, but not IL-8 and TNF-a mRNA (Fig. 2E). Moreover, the ELISA showed that pretreatment with 12.5 and 25 μg/mL STE significantly reduced the levels of IL-6 in HaCaT cells (Fig. 2F and Table 1).

STE suppressed the level of phosphorylated ERK in NHDF cells

The expression of proinflammatory cytokines, such as TNF-α, IL-6 and IL-8, is regulated by the MAPK and NF-κB pathways. We therefore examined the effects of STE on MAPK and NF-κB signaling and the role of these pathways in TNFα-stimulated NHDF cells. The effects of STE on the MAPK signaling pathway were determined by measuring the levels of phosphorylated p38, ERK and JNK proteins. Treatment with STE significantly and dose-dependently reduced phospho-ERK, but not phospho-p38 and phospho-JNK, expression (Fig. 3). To further explore its molecular mechanisms, the effects of STE on IκBα degradation and IκBα phosphorylation were assessed. STE did not inhibit total-IκBα degradation, although IκBα phosphorylation was slightly inhibited at the concentration of 100 μg/mL (Fig. 3). Taken together, these results suggest that STE may regulate the secretion of TNF-α, IL-6 and IL-8 by inhibiting ERK phosphorylation, independent of the IκBα signaling pathway.

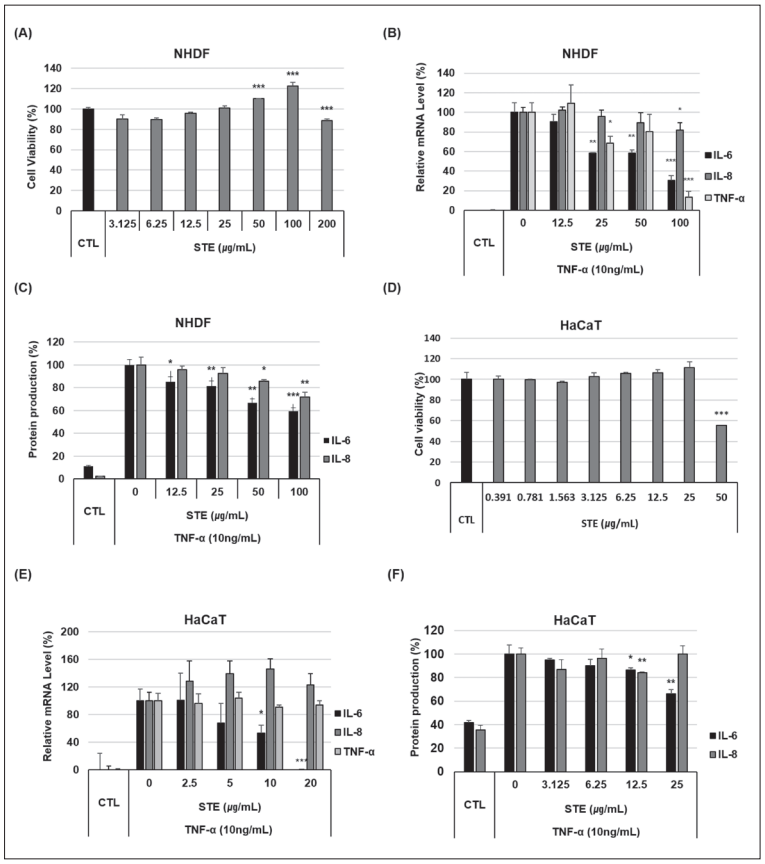

Fig. 2. Effect of STE on NHDF and HaCaT cell viability and production of cytokine mRNA and protein. **A** – Effect on NHDF cell viability. Cells were incubated with STE at 3.125~200 μg/mL for 48 h, and cell viability was assessed using CCK-8 assays. **B** – Effect on IL-6, IL-8 and TNF-α mRNA levels in NHDF cells. Cells were pretreated with 12.5~100 μg/mL STE for 3 h and stimulated with 10 ng/mL TNF-α for 24 h. mRNA levels were determined by real-time PCR and normalized relative to those of GAPDH. **C** – Effect on the production of IL-6 and IL-8 proteins by NHDF cells. Cells were pretreated with 12.5~100 μg/mL STE for 3 h and stimulated with 10 ng/mL TNF-α for 48 h. Protein production was determined by ELISA. **D** – Effect on HaCaT cell viability. Cells were incubated with STE at 0.3~50 μg/mL for 48 h, and cell viability was assessed by the CCK-8 assay. **E** – Effect on IL-6, IL-8 and TNF-α mRNA levels in HaCaT cells. Cells were pretreated with 2.5~20 μg/mL STE for 3 h and stimulated with 10 ng/mL TNF-α for 24 h. mRNA levels were determined by real-time PCR and normalized relative to those of GAPDH. **F** – Effect on the production of IL-6 and IL-8 proteins by HaCaT cells. Cells were pretreated with 3.125~250 μg/mL STE for 3 h and stimulated with 10 ng/mL TNF-α for 48 h. The results are presented as the mean expression level obtained from three independent experiments. Data are presented as the mean±SD. ***P<0.001 compared with the control group (A and D) and *P<0.05, **P<0.01, ***P<0.001 compared with cells treated with TNF-α alone (B, C, E and F).

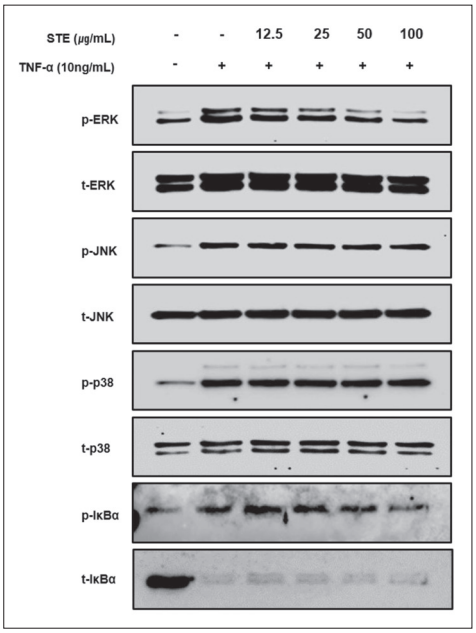

Fig. 3. Effect of STE on the levels of total and phosphorylated ERK, JNK, p38 and IκBα in NHDF cells. Cells were pretreated with 12.5, 25, 50 and 100 μg/mL STE for 3 h and stimulated with 10 ng/mL TNF-α for 10 min. Western blot analysis was conducted to determine the protein levels of total (t-) and phosphorylated (p-) ERK, JNK, p38 and IκBα.

STE has the DPPH radical scavenging ability

DPPH assays were performed to determine whether STE has antioxidative properties. At the concentration range of 1.563~100 μg/mL, STE dramatically suppressed DPPH radical activity in a dose-dependent manner (Fig. 4). At a concentration of 10 μg/mL, ascorbic acid (the positive control) showed high DDPH radical scavenging activity of 86±0.21%, similar to that of 100 μg/mL STE, which had a DDPH radical scavenging activity of 86±0.27%. Treatment with STE turned the color of the plate yellow, indicating that STE has a ROS scavenging effect.

DISCUSSION

Vitiligo is a depigmenting disease characterized by the appearance of white patches on the skin. The primary therapeutic strategy consists therefore of treatment with agents that enhance the proliferation and activity of melanocytes, thereby inducing melanin production. In addition, antiinflammatory agents and antioxidants play important roles in the treatment of vitiligo. Vitiligo

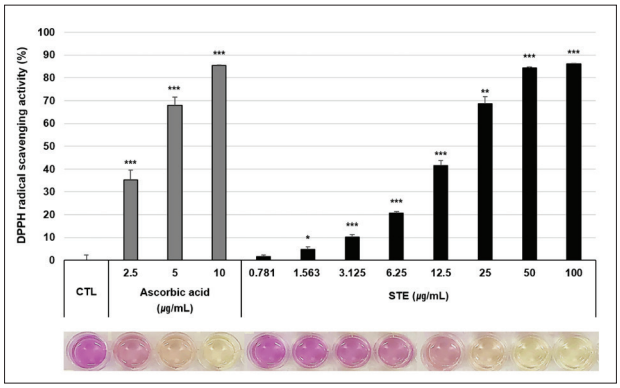

Fig. 4. DPPH radical scavenging ability of STE. Ascorbic acid was used as a positive control. The results are presented as the mean expression level obtained from three independent experiments. Data are presented as mean±SD. *P<0.05, **P< 0.01, ***P<0.001 compared with the control group.

may be treated by phototherapy with UVA and UVB light, which enhance the migration and proliferation of all melanocytes, promote a favorable environment for melanocyte growth and suppress autoimmunity. Helium neon laser treatment, with a mechanism of action similar to other types of phototherapy, has been shown to be effective in patients with localized vitiligo.

S. commixta is a formulation used in traditional Korean medicine to darken white or gray hair and to treat several inflammatory diseases, suggesting that its use may improve outcomes in patients with vitiligo [14]. Thus, the present study assessed the *in vitro* efficacy and pharmacological effects of STE in melanocytes, fibroblasts, and keratinocytes. To our knowledge, the present study is the first to report the efficacy of STE in melanocytes. Although we found that STE enhanced the melanin content in B16 cells, the present study did not evaluate its mechanism of action. Previous studies have reported that cAMP induces cAMP-response element binding protein (CREB) phosphorylation, a frequent indicator of the upregulation of the basic microphthalmia-associated transcription factor (MITF), which is important for melanin production. MITF critically regulates melanin production-related genes, such as those encoding tyrosinase and tyrosinase-related proteins (TRP)-1 and -2. Further studies are needed to evaluate the molecular mechanism by which STE enhances melanin synthesis, both in mouse melanocytes such as B16 and in human melanocytes [15].

STE also has antiinflammatory properties. In a previous report, STE was found to significantly suppress UVB-induced upregulation of matrix metalloproteinases (MMP)-1, MMP-2 and MMP-3 expression through MAPK inactivation [12]. In addition, the water extract of *S. commixta* was shown to be a potent antiinflammatory agent, suppressing the inflammatory signaling cascade composed of Src, Syk and NF-κB [1]. In the current study, we also observed that STE inhibited the expression and production of proinflammatory cytokines such as IL-6, IL-8 and TNF-α. These data suggested a possibility that STE may have anti-vitiligo efficacy through its antiinflammatory properties in skin cells, although STE showed more potent antiinflammatory effect in NHDF than in HaCaT cells.

The MAPK signaling cascade transmits a variety of extracellular signals that regulate cellular responses related to proliferation, differentiation and death. This cascade includes three separate sub-pathways, involving extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 [10]. Keratinocytes synthesize cytokines such as TNF-α, IL-6 and transforming growth factor-β (TGF-β), which are paracrine inhibitors of human melanocyte proliferation and melanin production. ERK regulates the production of proinflammatory cytokines, such as TNF-α and IL-6, as well as other signaling molecules, including prostaglandins and nitrogen oxides [9]. Abnormal ERK activation has been observed in human inflammatory diseases [16]. Pharmacological inhibition of the ERK pathway was found to reduce TNF-α production by leukocytes [17]. In addition, NF-κB is involved in inflammatory responses and cell proliferation [18]. In most cells, NF-κB protein dimers bind to the inhibitor κBα (IκBα), which suppresses NF-κB-induced activation [19]. NF-κB signaling is also related to vitiligo [20]. Our study confirmed that ERK phosphorylation is reduced in STE-treated NHDFs [21]. However, STE has no effect on TNF-α-induced IκBα degradation. The data suggested that STE reduced inflammatory cytokines through ERK inactivation in NHDFs.

Oxidative stress is an important cause of vitiligo. Furthermore, increased levels of reactive oxygen species (ROS) in melanocytes may cause apoptosis, resulting in the release of aberrant proteins, which can serve as autoantigens and induce autoimmunity [22-23]. Therefore, antioxidative potential is thought

to be an important factor in the treatment of vitiligo. The present study found that STE had DPPH radical scavenging ability, thereby protecting melanocytes from oxidative stress.

Recently, several researchers have reported the composition of *S. commixta* extract. The chemical β-sitosteryl-3-O-β-glucopyranoside isolated from the bark of *S. commixta* suppressed the production of NO and preinflammation by downregulating the NF-κB pathway in lipopolysaccharide (LPS)-induced RAW 264.7 cells, a murine monocyte/macrophage cell line [24]. In addition, two new phenolic glycosides were isolated from *S. commixta,* showing that these compounds inhibited the production of proinflammatory molecules. [24-25]. In our further endevours, we will focus on identifying the functional compounds for the treatment of vitiligo in STE, besides the compounds mentioned above.

Collectively, our findings reveal for the first time that STE may be effective in the treatment of vitiligo through melanin synthesis, antiinflammation and antioxidative efficacy.

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Author contributions: DW Jung and CS Lee designed the study. DW Jung performed most of the experiments. SJ Lim supported the experiments. DW Jung and CS Lee analyzed the data and contributed to the writing of the manuscript.

Conflicts of interest disclosure: The authors declare no conflict of interest.

REFERENCES

1. Yu T, Lee YJ, Jang H-J, Kim AR, Hong S, Kim TW, Kim M-Y, Lee J, Lee YG, Cho JY. Anti-inflammatory activity of Sorbus commixta water extract and its molecular inhibitory mechanism. J Ethnopharmacol. 2011;134(2):493-500. https://doi.org/10.1016/j.jep.2010.12.03
2. Taïeb A, Picardo M. The definition and assessment of vitiligo: a consensus report of the vitiligo european task force. Pigment Cell Res. 2007;20(1):27-35. https://doi.org/10.1111/j.1600-0749.2006.00355.x
3. Alikhan A, Felsten LM, Daly M, Petronic-Rosic V. Vitiligo: A comprehensive overview. J Am Acad Dermatol. 2011;65(3):473-91. https://doi.org/10.1016/j.jaad.2010.11.061
4. Allam M, Riad H. Concise review of recent studies in vitiligo. Qatar Med J. 2014;2013(2):1-19. https://doi.org/10.5339/qmj.2013.10
5. Boniface K, Seneschal J, Picardo M, Taïeb A. Vitiligo: Focus on clinical aspects, immunopathogenesis, and therapy. Clin Rev Allergy Immunol. 2017;54(1):52-67. https://doi.org/10.1007/s12016-017-8622-7
6. Kundu RV, Mhlaba JM, Rangel SM, Poole ICL. The convergence theory for vitiligo: A reappraisal. Exp Dermatol. 2018;28(6):647-55. https://doi.org/10.1111/exd.13677
7. Choi H, Kim K, Han J, Choi H, Jin SH, Lee EK, Shin DW, Lee TR, Lee A-Y, Noh M. Kojic acid-induced IL-6 production in human keratinocytes plays a role in its anti-melanogenic activity in skin. J Dermatol Sci. 2012;66(3):207-15. https://doi.org/10.1016/j.jdermsci.2012.03.002
8. Manga P, Elbuluk N, Orlow SJ. Recent advances in understanding vitiligo. F1000Research. 2016;5:2234. https://doi.org/10.12688/f1000research.8976.1
9. Laddha NC, Dwivedi M, Begum R. Increased tumor necrosis factor (TNF)-α and its promoter polymorphisms correlate with disease progression and higher susceptibility towards vitiligo. PLoS One. 2012;7(12):e52298. https://doi.org/10.1371/journal.pone.0052298
10. Sabio G, Davis RJ. TNF and MAP kinase signalling pathways. Semin Immunol. 2014;26(3):237-45. https://doi.org/10.1016/j.smim.2014.02.009
11. Rashighi M, Harris JE. Vitiligo pathogenesis and emerging treatments. Dermatol Clin. 2017;35(2):257-65. https://doi.org/10.1016/j.det.2016.11.014
12. Xuan SH, Park YM, Park SH, Jeong HJ, Park SN. Suppression of ultraviolet B-mediated matrix metalloproteinase generation by Sorbus commixta twig extract in human dermal fibroblasts. Photochem Photobiol. 2018;94(2):370-7. https://doi.org/10.1111/php.12868
13. Salducci M, André N, Guéré C, Martin M, Fitoussi R, Vié K, Cario-André M. Factors secreted by irradiated aged fibroblasts induce solar lentigo in pigmented reconstructed epidermis. Pigment Cell Res. 2014;27(3):502-4. https://doi.org/10.1111/pcmr.12234
14. Felsten LM, Alikhan A, Petronic-Rosic V. Vitiligo: A comprehensive overview. J Am Acad Dermatol. 2011;65(3):493-514. https://doi.org/10.1016/j.jaad.2010.10.043
15. Jian D, Jiang D, Su J, Chen W, Hu X, Kuang Y, Xie H, Li J, Chen X. Diethylstilbestrol enhances melanogenesis via cAMP-PKA-mediating up-regulation of tyrosinase and MITF in mouse B16 melanoma cells. Steroids. 2011;76(12):1297-304. https://doi.org/10.1016/j.steroids.2011.06.008
16. Dumitru CD, Ceci JD, Tsatsanis C, Kontoyiannis D, Stamatakis K, Lin J-H, Patriotis C, Jenkins NA, Copeland NG, Kollias G, Tsichlis PN. TNF-α Induction by LPS is regulated post transcriptionally via a Tpl2/ERK-Dependent Pathway. Cell. 2000;103(7):1071-83. https://doi.org/10.1016/S0092-8674(00)00210-5
17. Schett G, Tohidast-Akrad M, Smolen JS, Schmid BJ, Steiner C-W, Bitzan P, Zenz P, Redlich K, Xu Q, Steiner G. Activation, differential localization, and regulation of the stress-activated protein kinases, extracellular signal-regulated kinase, c-Jun N-terminal kinase, and p38 mitogen-activated

protein kinase, in synovial tissue and cells in rheumatoid arthritis. Arthritis Rheum. 2000;43(11):2501-12. https://doi.org/10.1002/1529-0131(200011)43:11<2501::AID-ANR18>3.0.CO;2-K
18. Trotta R, Kanakaraj P, Perussia B. Fc gamma R-dependent mitogen-activated protein kinase activation in leukocytes: a common signal transduction event necessary for expression of TNF-alpha and early activation genes. J Exp Med. 1996;184(3):1027-35. https://doi.org/10.1084/jem.184.3.1027
19. Oeckinghaus A, Ghosh S. The NF- B Family of transcription factors and its regulation. Cold Spring Harb Perspect Biol. 2009;1(4):a000034. https://doi.org/10.1101/cshperspect.a000034
20. Moynagh PN. The NF-κB pathway. J Cell Sci. 2005;118(20):4589-92. https://doi.org/10.1242/jcs.02579
21. Wan J, Lin F, Zhang W, Xu A, Degiorgis J, Lu H, et al. Novel approaches to vitiligo treatment via modulation of mTOR and NF-κB pathways in human skin melanocytes. Int J Biol Sci. 2017;13(3):391-400. https://doi.org/10.7150/ijbs.17318
22. Xie H, Zhou F, Liu L, Zhu G, Li Q, Li C, Gao T. Vitiligo: How do oxidative stress-induced autoantigens trigger autoimmunity? J Dermatol Sci. 2016;81(1):3-9. https://doi.org/10.1016/j.jdermsci.2015.09.003
23. Bickers DR, Athar M. Oxidative stress in the pathogenesis of skin disease. J Invest Dermatol. 2006;126(12):2565-75. https://doi.org/10.1038/sj.jid.5700340
24. Yang G, An H-J. β-sitosteryl-3-O-β-glucopyranoside isolated from the bark of Sorbus commixta ameliorates pro-inflammatory mediators in RAW 264.7 macrophages. Immunopharmacol Immunotoxicol. 2013;36(1):70-7. https://doi.org/10.3109/08923973.2013.866956
25. Kim CS, Oh J, Subedi L, Kim SY, Choi SU, Lee KR. Two new phenolic glycosides from Sorbus commixta. Chem Pharm Bull. 2018;66(8):839-42. https://doi.org/10.1248/cpb.c18-00280