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Review

Prominent Anti-UV Activity and Possible Cosmetic Potential of Lignin-carbohydrate Complex

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Abstract. *This review article summarizes the recent progress of ultraviolet rays (UV) protective substances, including our original reports. We have established a simple assay method for the determination of anti-UV activity that can be applicable to any kind of adherent cells. This method provides information of both anti-UV activity and cytotoxicity of any kind of samples even though those samples contain unknown amounts of test compounds. We found that lignin-carbohydrate complex (LCC) showed one- or two-order higher anti-UV activity compared to well-known lower molecular weight polyphenols and hot-water extracts of Kampo medicines and tea leaves. Among synthetic compounds, water-soluble azulenes showed the highest anti-UV activity. LCC showed additive or synergistic anti-UV activity with vitamin C. Alkaline extract of Sasa senanensis Rehder leaves (SE), an LCC-rich over-the-counter (OTC) drug, also showed potent antiviral and vitamin C-synergized radical scavenging activity. SE has been utilized to manufacture tooth paste, soap and gel cosmetic to increase the level of quality of life (QOL).*

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Ultraviolet rays (UV) are classified into UVA (400–315 nm), UVB (315–280 nm) and UVC (<280 nm), depending on the wavelength. UVA and UVB pass through the ozonosphere and reach the ground of earth's surface, whereas UVC cannot pass through the air due to absorption. Ninety-nine percent of UV that reaches the ground is UVA. Moderate doses of UV have been reported to exert several favorable effects, such as sterilization and disinfection (1), induction of vitamin D biosynthesis (2) and stimulation of the metabolism. On the other hand, an excessive UV radiation, absorbed by epidermis, produces reactive oxygen species (ROS) and causes various cutaneous disorders (3). Guanine, the most susceptible DNA base, is oxidized to 7,8-dihydro-8-oxoguanine upon UV-irradiation and triggers the transversion of G:C to T:A (4-5). High doses of UV irradiation have been shown to induce apoptotic cell death in human myelogenous leukemia cell lines but, on the contrary, non-apoptotic cell death in human T-cell leukemia, erythroleukemia, glioblastoma (6), oral squamous cell carcinoma (OSCC) cell lines and human normal oral cells (gingival fibroblasts, pulp cells and periodontal ligament fibroblasts) (7).

In vitro experiments are very useful to screen various natural products for lifelong protection of skin under unconscious sun UV exposure. A combination of rosemary and citrus bioflavonoids extracts inhibited the harmful effects of UV on human HaCaT keratinocytes, a spontaneously transformed aneuploid immortal keratinocyte cell line derived from adult human skin, by decreasing UVB-induced intracellular ROS and preventing DNA damage (8). Major green tea component, (–)-epigallocatechin-3-gallate (EGCG), has been shown to dose-dependently protect human lung fibroblasts, skin fibroblasts and epidermal keratinocytes, as well as peripheral blood cells from UV-induced DNA damage, as assessed by the alkaline comet assay (9). Based

on the quenching effect of azelaic acid, a C9 dicarboxylic acid, hydroxyl radical was identified as one of the major culprit of UV-induced cytotoxicity (10).

Recently, plant extracts (11), agronomical disposable wastes (12) and plant cell cultures (13) are valuable sources for cosmetics. However, previous studies have not provided the chemotherapeutic index and, therefore, which material is the most potent and less toxic is not clear. Based on these backgrounds, we have established a simple method that can evaluate the activity of the compound/extract to protect cells from UV-induced injury (referred to as 'anti-UV activity') and the accompanied cytotoxicity to generate the selectivity index (14). This review is the summary of our global analysis of anti-UV activity of plant extracts, natural products and synthetic compounds.

Development of Assay for Anti-UV Activity

It is most reasonable to use UVA that reaches the ground at much higher percentage rather than UVC. However, it is possible that UVC might reach the ground due to the stratospheric ozone depletion caused by continuous release of carbon monoxide (vehicle exhaust) (15), halogenated gas (16) and nitrous oxide (17) in quantities that nobody knows how much such leakage affects the function of the human body. Furthermore, UVA and UVB are known to induce insufficient cytotoxicity for the calculation of the 50% cytotoxic concentration (CC_{50}) value (18). Based on these backgrounds, we have adopted the UV-C irradiation that has the shorter wavelength and, thus, the higher energy than UV-A and UV-B, producing the reproducible and quantitative cytotoxicity in most of the cells.

The outline of our original assay method of anti-UV activity is illustrated in Figure 1A. The medium of cells in the 96-microwell plates was completely discarded and replaced with phosphate-buffered saline without calcium and magnesium (PBS (–)) to determine any UV-absorbing substance(s). Different concentrations of samples were then added to the cell lines (mostly UV-sensitive human oral squamous cell carcinoma HSC-2 and Ca9-22 and, in some cases, human skin immortal keratinocyte HaCaT and human fibrosarcoma HT1080) and all plates (on which cells are attached) were immediately placed at 20.5 cm just below from a UV lamp on the clear bench (Hitachi straight-tube germicidal lamp, GL-15, 436×25.5 mm, rated power consumption 15W, ultraviolet output 4.4 W, wavelength 253.7 nm) (attached to clean bench) and exposed to UV irradiation for 1 min. We used a 1-min exposure time since this provided the highest anti-UV activity and the prolonged exposure in PBS(–) reduced cell viability (14). The radiation dose emitted from the UV lamp, measured by the Digital UV Meter (YK-35UV; Sato Shoji Co., Ltd., Kawasaki, Japan), was 0.788±0.042 mW/cm^2, with a radiation dose reaching the cells of 0.093±0.012 mW/cm^2. After UV irradiation, the media were replaced with fresh DMEM plus 10% FBS and cells were then cultured at 37˚C in an 5% CO_2 incubator until 48 hours after the start of irradiation (Figure 1A).

From the dose-response curve without UV irradiation (control), the CC_{50} of the sample is obtained. UV irradiation reduced cell viability, usually up to 0~20% of the control level. Addition of increasing concentrations of anti-UV substances during UV-irradiation, such as plant extracts, natural products and synthetic compounds, reversed the reduced cell number to various extents. From the dose-response curve, the 50% effective concentration (that recovered the viable cell number to 50% of control level) (EC_{50}) of the sample is obtained. The selectivity index (SI) (chemotherapeutic index or safety margin) is calculated by the following equation: $SI=CC_{50}/EC_{50}$ (one example is shown in Figure 1B, left panel). However, when anti-UV activity of test samples is very weak or highly cytotoxic, it is impossible to calculate the EC_{50} accurately. In that case, the anti-UV activity (defined as SI value) is expressed by using the inequality sign (one example is shown in Figure 1B, right panel).

Since the CC_{50} values are usually comparable with or without UV irradiation (Figure 1B, left panel), this assay method can provide the SI value of the samples that have unknown concentrations of anti-UV substances (19).

Screening of Anti-UV Substances

Due to the lack of information of quantitative anti-UV activity in previous publications, we have compared the relative anti-UV activity of nine groups of plant extracts, natural products and synthetic compounds. The results of previous and ongoing research are summarized in Table I.

Lignin-carbohydrate complex (LCC) (Group A). There are three major classes of polyphenols in the land-plant kingdom: lignin-carbohydrate complex (LCC), tannins and flavonoids. As compared with tannins and flavonoids, LCCs exist as amorphous large structures in the plant cell wall, making it difficult to determine the complete structure of the extracted forms of LCC (20). Among these three groups of polyphenols, LCC, prepared by sequential alkaline extraction and acid precipitation, from pine cone (*Pinus parviflora* Sieb. et Zucc., *Pinus taeda* L and *Pinus elliottii* var. elliottii) and pine seed shell (*Pinus parviflora* Sieb. et Zucc.) (21), as well as extracts of *Lentinus edodes* mycelia and *Sasa senanensis* Rehder leaves (19), showed an excellent anti-UV activity (SI=25.6~41.9). Their anti-UV activity was comparable to that of vitamin C (SI=42.4) but much higher than that of resveratrol (SI<1) (Figure 1B), both known as protective compounds.

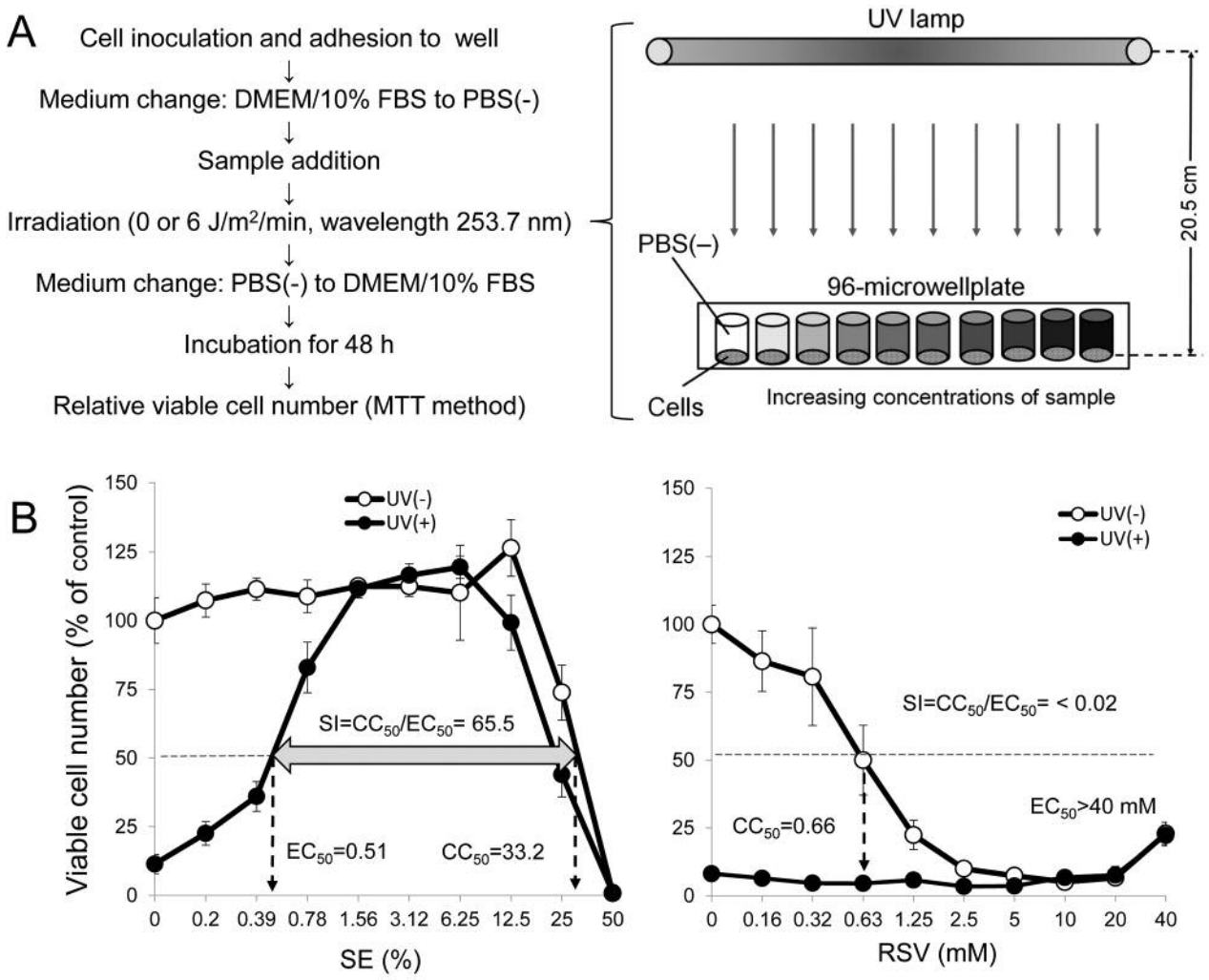

Figure 1. *(A) Schematic diagram of measurement of anti-UV activity. (B) Calculation method of SI values. HaCaT cells, spontaneously transformed keratinocytes from histologically normal skin, were exposed or not for 1 min to UV in the presence of increasing concentrations of alkaline extract of Sasa senanensis Rehder (SE) leaves and resveratrol (RSV). SI values were determined by the following equation: $SI=CC_{50}/EC_{50}$. The experimental protocol has been outlined in the text.*

Chemically-modified glucans (Group B). *N,N*-Dimethylaminoethyl (DMAE)-laminarin, DMAE-pullulan, DMAE-dextran and paramylon sulfate showed no anti-UV activity. Taken together with the results described in section 3.1 of a previous publication (21), anti-UV activity of LCC seems likely to be derived from the lignin moiety composed of highly polymerized phenylpropenoids rather than the carbohydrate moiety.

Lower-molecular weight polyphenols (Group C). Resveratrol, a stilbenoid known for its anti-aging effect, showed very low anti-UV activity (SI<1), regardless of the target cells (unpublished data). Similarly, epigallocatechin gallate (a major component of green tea) (SI=7.7) and gallic acid (a component unit of tannin) (SI=5.4) showed relatively lower SI values (21). Luteolin glycosides (luteolin 6-C-β-D-glucoside, isolated from *Sasa senamensis* Rehder luteolin 7-O-β-D-glucoside and luteolin 6-C-α-D-glucoside) showed certain anti-UV activity (SI=2.8~23.5) (22).

Antioxidants (Group D). Vanillin and sodium ascorbate (vitamin C) (21) showed potent anti-UV activity (SI=63.8 and 42.4, respectively). On the other hand, curcumin, Arturmerone, *N*-acetyl-L-cysteine and catalase showed no anti-UV activity (SI<1) (21). These data are consistent with previous reports where superoxide dismutase (scavenges superoxide anion radicals) and catalase (degrades hydrogen peroxide) did not show any protective effect on UV-induced cytotoxicity (10).

Table I. *Anti-UV activity of natural products and synthetic compounds*.

	Samples (n=number of samples)	Target cells	Anti-UV activity (SI)	References
Group A Lignin-carbohydrate complex	Pine cone extract (n=3)	HCS-2	33.4±7.4 (24.8~38.1)	19
	Pine seed shell extract		25.6	
	Lentinus edodes mycelia extract		41.9	
	Sasa senanensis Rehder leaves		38.5	
	Alkali-lignin (commercial)		>61.5	
Group B Chemically modified glucans	DMAE-laminarin	HSC-2	<1	19
	DMAE-pullulan		<1	
	DMAE-dextran		<1	
	Paramylon sulfate		<1	
Group C Lower molecular weight polyphenols	Reseveratrol	Ca9-22	<1	Unpublished data
		HaCaT	<0.02	
		HT1080	<0.01	
	Epigallocatechin gallate	HSC-2	7.7	20
	Gallic acid		5.4	
	Luteolin 6-*C*β-D-glucoside		>2.8	22
	Luteolin 7-*O*-β-D-glucoside		7.3	
	Luteolin 6-*C*-α-D-glucoside		>7.1	
	Tricin		23.5	
Group D Antioxidants	Vanillin	HSC-2	63.8	19
	Sodium ascorbate (vitmin C)	HSC-2	42.4	20
	Curcumin		<1	
	Ar-turmerone		<1	
	N-Acetyl-L-cysteine		<1	
	Catalase		<1	
Group E Synthetic compounds	Azulene-related compounds (n=5)	HSC-2	<1	7
	Troplolone-related compounds (n=11)	HSC-2	<1	14
	2-Aminotropones (n=20)	HSC-2	3.5±1.6 (0.1~5.6)	23
	Water-soluble azulenes (n=8)	HSC-2	35.5±21.6 (4.5~65.6)	24
Group F Methanol extract of plants	Odontioda Marie Noel 'Verano'	HSC-2	<1	25
Group G Alkaline extract of plants	*Sasa senanensis* Rehder leaves	HSC-2	19.5	22
		HaCaT	65.5	Unpublished data
		HT1080	51.9	
	Green tea leaves	HSC-2	>10.4	28
	Oolong tea leaves		>7.4	
	Orange flower		>4.0	
Group H Hot water extracts	Green tea leaves	HSC-2	3.4	20
	Black tea leaves		<1	
	Jasmine tea leaves		<1	
	Coffee		9.6	
	Oolong tea leaves (Pet bottle)		<1	
	Green tea leaves (Pet bottle)		1.6	
	Oh-ki tea leaves (Pet bottle)		<1	
	Burley tea leaves (Pet bottle)		<1	
Group I Kampo medicines	Mixtures (n=10)	HSC-2	2.4±1.8 (1~4.9)	29
	Constituent plant extracts (n=25)		1.4±1.6 (1~8)	
	Mixtures (n=10)	HaCaT	15.6±11.1 (4.4~34.8)	30
	Constituent plant extracts (n=25)		10.6±9.3 (0.1~38.0)	

Synthetic compounds (Group E). Azulene, produced by heating, is present in limited numbers of organisms. On the other hand, numerous derivatives of tropolone are present in the natural kingdom. Five azulene and tropolone-related compounds, such as benzo[*b*]cyclohepta[e][1,4]thiazine, 6,8-dibromobenzo[*b*]cyclohepta[e][1,4]thiazine, benzo[*b*]cyclo hepta[e][1,4]oxazin-6(11*H*)-one and 3-methyl-1-trichloro-acetylazulene, 3-ethyl-1-trichloroacetylazulene showed very weak anti-UV activity (SI<1). Due to poor water-solubility, much higher (millimolar orders) concentrations of these samples could not be applied (7).

Eleven azulene and tropolone-related compounds, such as 2,3-dimethyl-1-trichloroacethylazulene, 1,3-ditrichloroacetyl-4,6,8-trimethylazulene, 6,8,10-tribromobenzo[*b*]cyclohepta [*e*][1,4]oxazine, 6-bromo-2-chlorobenzo[*b*]cyclohepta[*e*][1,4] oxazine, 7-bromo-2-(4-hydroxyanilino)tropone, 2-(2-hydro-xyanilino)-4-isopropyltropone, benzo[*b*]cyclohepta[*e*][1,4] thiazine, 6,8-dibromobenzo[*b*]cyclohepta[*e*][1,4]thiazine, benzo [*b*]cyclohepta[*e*][1,4]oxazin-6(11*H*)-one, 3-methyl-1-trichloroa-cetylazulene and 3-ethyl-1-trichloroacetylazulene showed no anti-UV activity (SI<1) due to their insolubility (14).

Twenty 2-aminotropones, such as 2-*N*-methylaminotropone, 2-*N*-ethylaminotropone, 2-*N*-*n*-propylaminotropone, 2-*N*-*n*-butylaminotropone, 2-*N*-*n*-pentylaminotropone, 2-*N*-*n*-hexylaminotropone, 2-*N*-(2-hydroxyethyl)aminotropone, 2-*N*-(3-hydroxypropyl)aminotropone, 2-*N*-(4-hydroxybutyl)amino-tropone, 2-*N*-(5-hydroxypentyl)aminotropone, 2-*N*-methyla-mino-5-isopropyltropone, 2-*N*-ethylamino-5-isopropyltropone, 2-*N*-*n*-propylamino-5-isopropyltropone, 2-*N*-*n*-butylamino-5-isopropyltropone, 2-*N*-*n*-pentylamino-5-isopropyltropone, 2-*N*-*n*-hexylamino-5-isopropyltropone, 2-*N*-(2-hydroxyethyl)amino-5-isopropyltropone, 2-*N*-(3-hydroxypropyl)amino-5-isopro-pyltropone, 2-*N*-(4-hydroxybutyl)amino-5-isopropyltropone and 2-*N*-(5-hydroxypentyl)amino-5-isopropyltropone showed some anti-UV activity (SI=0.1~5.6) (23).

To overcome water-insolubility, we have synthesized water-soluble azulene derivatives, such as sodium 3-methylazulene-1-sulfonate, sodium 3-ethylazulene-1-sulfonate, sodium 3-propylazulene-1-sulfonate, sodium 7-isopropyl-3-methylazu-lene-1-sulfonate, sodium 7-isopropyl-3-ethylazulene-1-sulfonate, sodium 7-isopropyl-3-propylazulene-1-sulfonate, sodium 3-hexylazulene-1-sulfonate, sodium 7-isopropyl-3-heptylazulene-1-sulfonate and disodium azulene 1,3-disulfonate, testing their possible anti-UV activity. As expected, these newly synthesized water-soluble azulenes showed much higher anti-UV activity (SI=4.5~65.6) (24).

Methanol extract of plants (Group F). Methanol extracts of *Odontioda* Marie Noel 'Verano' and its subfractions obtained by sequential extraction with *n*-hexane, ethyl acetate and n-butanol, as well as residual water layer, showed no anti-UV activity possibly due to the presence of cytotoxic substances in the extracts (25).

Alkaline extracts of plants (Group G). Alkaline extracts of *Sasa senanensis* Rehder leaves (OTC drug named as "SASA-health", SE) showed anti-UV activity comparable to LCC (22, 26, 27). It should be noted that SE's anti-UV activity was more pronounced when skin-derived cells (HaCaT) were used as target cells (SI=65.5) (manuscript in preparation) as opposed to HSC-2 oral squamous cell carcinoma cells (SI=19.5) (27). Likewise, alkaline extracts of green tea leaves, oolong tea leaves and orange flower showed one order lower anti-UV activity (SI=4.0~10.4) than LCC (28).

Hot-water extracts (Group H). Hot-water extracts of leaves of green tea (SI=1.6~3.4), black tea (SI<1), jasmine tea (SI<1), oh-ki tea (SI<1) and burley tea (SI<1) showed essentially no anti-UV activity, regardless of using freshly prepared extracts or commercially available beverages contained in PET plastic bottles, except coffee (SI=9.6) (19).

Kampo medicines (Group J). Ten Kampo medicines (Byakkoka-ninjinto, Hangeshashinto, Hotyuekkito, Juzentaihoto, Kikyoto, Ninjinyoeito, Rikkosan, Saireito, Shosaikoto and Unseiin) showed much lower anti-UV activity (S=1~4.9), while their constituent plant extracts (*Alisma* rhizoma and *Asiasarum* root, *Astragalus* root, *Atractylodes Lancea* rhizoma, *Bupleurum* root, *cimicifuga* rhizoma, cinnamon bark, *Cnidium* Rhizoma, Coptis rhizoma, Gardenia fruit, ginger, ginseng, glycyrrhiza, Japanese angelica root, Japanese gentian, Jujube, Peony root, Phellodendron bark, Pinellia tuber, Platycodon root, Polyporus sclerotium, Poria sclerotium, Rehmannia root, Saposhnikovia root and Scutellaria root) showed relatively lower anti-UV activity (SI=1~8) (29).

Generally, change of target cells from HSC-2 to HaCaT cells increased SI values approximately 6~8-fold and a valid correlation of these two systems was confirmed (R^2=0.9118) (30).

Unique Properties of Alkaline Extracts of *Sasa senanensis Rehder* Leaves

Identification of anti-UV principle as LCC. We found that alkaline extracts of *Sasa senanensis* Rehder leaves (SE) showed potent anti-UV activity as compared with LCC (Table I).

By high-performance liquid chromatography (HPLC), we identified *p*-coumaric acid derivatives as one of the anti-UV substances, albeit its very low yield from SE (yield=0.39%) (26) (Figure 2A), as compared with LCC (yield=40.9%) (31). However, the complete chemical structure of this purified material could not be determined due to the presence of many degradation products after alkaline treatment. We have purified, as anti-UV substances from SE, four luteolin glycosides (luteolin 6-C-β-D-glucoside, luteolin 7-O-β-D-glucoside, luteolin 6-C-α-D-glucoside) and tricin at the yield

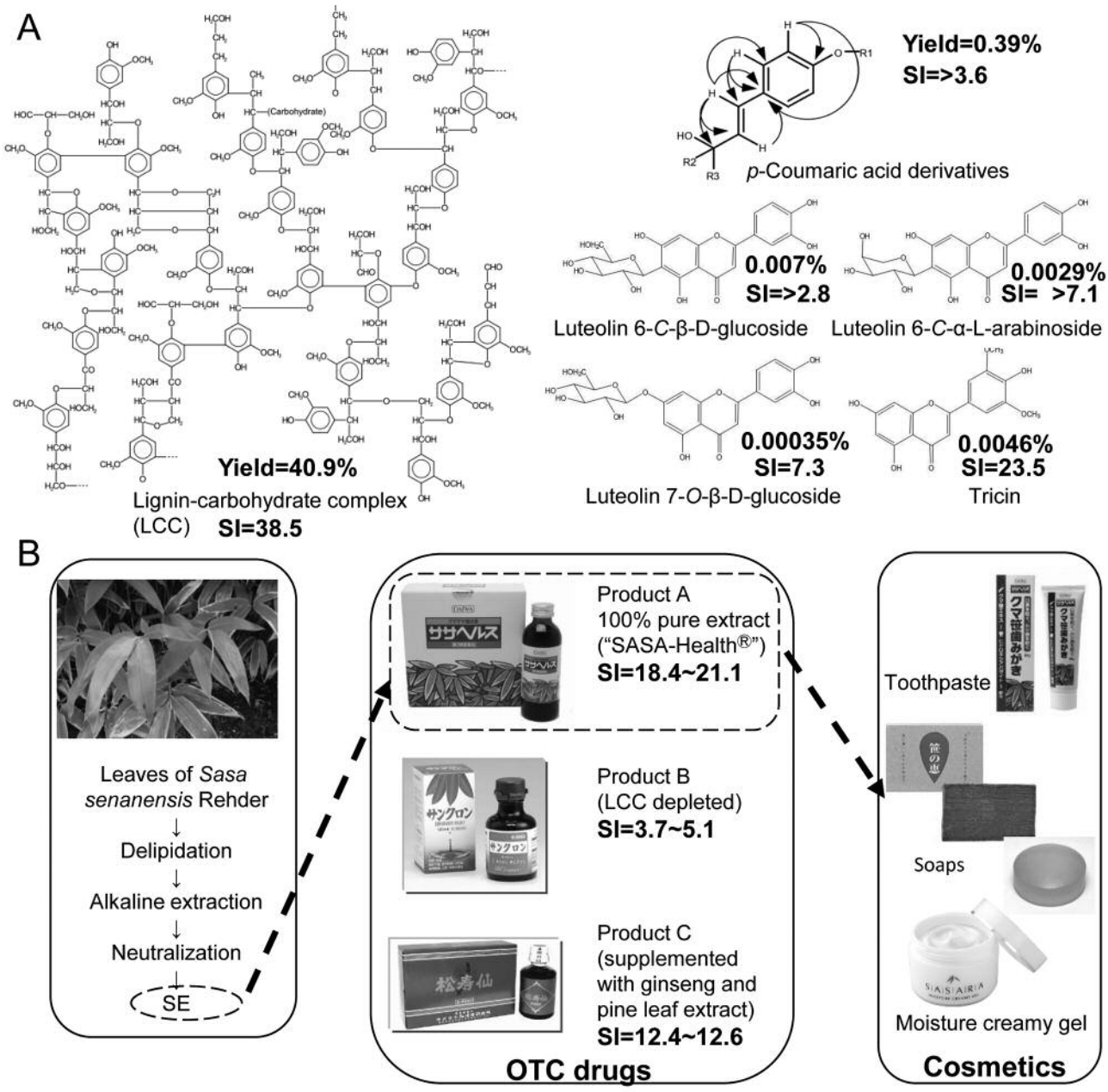

Figure 2. *Chemical structures of lignin-carbohydrate complex (LCC) (putative), p-coumaric acid derivatives, three luteolin glycosides and tricin isolated from alkaline extracts of Sasa senanensis Rehder (SE) leaves (A) and OTC drugs, as well as cosmetics manufactured from SE (B). Yield from SE (starting material) and anti-UV activity (defined as SI values) are shown.*

of 0.007, 0.00035, 0.0029 and 0.0046%, respectively. Based on the highest yield and greatest anti-UV activity, LCC was identified as a major anti-UV component in SE (Figure 2A). There are three commercially available liquid products of SE (products A, B and C). Product A (dry weight=58.8 mg/ml) is a pure SE composition containing Fe (II)-chlorophyllin, whereas products B (dry weight=77.6 mg/ml) and C (dry weight=27.0 mg/ml) contain Cu (II)-chlorophyllin and less LCC. In product B, approximately 80% of LCC was removed as precipitate during its production process. Product C is supplemented with ginseng and pine (*Pinus densiflora*) leaf extracts. Among these three products, product A (pure SE) showed the highest anti-UV activity (31), confirming that LCC is the major anti-UV substance in SE, and that no

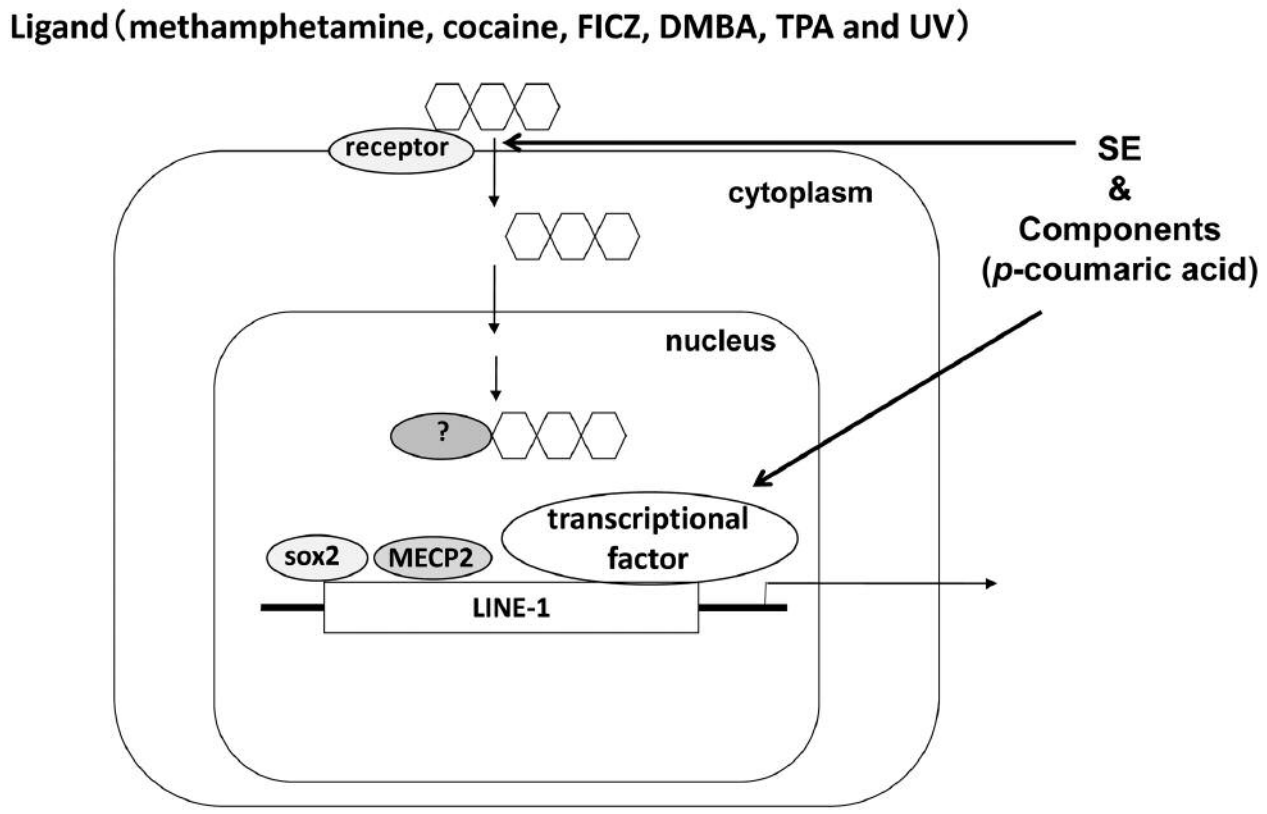

Figure 3. *Schematic diagram of putative action points of SE and its components, such as p-coumaric acid on the stimulation of LI gene expression.*

supplementation is necessary to increase its anti-UV activity. The granulated powder of SE manufactured from product A (SE-10) showed comparable or slightly higher anti-UV activity (32), suggesting that anti-UV activity of SE was not lost during the drying process. Taking advantage of this fact and using SE, we have manufactured several cosmetics, such as tooth paste (SE content=26.2% (w/v)), soap (SE content=3.6% (w/w)) and creamy gel (SE content=1.8% (v/v)) (Figure 2B).

Common properties with LCC. SE and Vitamin C (VC) scavenged superoxide anions (produced by hypoxanthin-xanthin oxidase reaction in the presence of 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) as DMPO-OOH) and hydroxyl radicals (produced by Fenton reaction in the presence of DMPO as DMPO-OH) synergistically (31). Moreover, the anti-UV activity of SE was enhanced by VC (SI=13.6 (0 mM VC)→20.5 (0.125 mM VC)→70.6 (0.25 mM VC) (27). Also, SE showed anti-HIV activity comparable to LCC prepared from various plants, which was one- or two-orders higher than that of lower molecular weight polyphenols (tannins, flavonoids and luteolin glycosides) (33).

Clinical study. We have reported that long-term treatment of a patient with lichenoid dysplasia with SE progressively reduced both the area of white streaks and the baseline levels of interleukin-6 and -8 in the saliva (34). Repeated experiments demonstrated that SE-containing toothpaste (SETP) significantly reduced halitosis but not the number of bacteria on the tongue (35). Likewise, oral intake of LCC-vitamin C-containing tablets significantly improved the symptom of patients infected with herpes simplex virus (36). It remains to be investigated whether SE-containing cosmetics effectively protects the skin from UV-induced damage.

Future direction

The present review clearly shows that SE, which contains significant amounts of LCC prepared from *Sasa senanensis* Rehder leaves by alkaline extraction, shows the highest anti-UV activity among natural polyphenols. Solubility is one of the important determinants of anti-UV activity of synthetic azulenes. It remains to be established whether the anti-UV activity is simply due to its UV absorbing property (37-38) or, rather, a modifying effect on redox-state in the cell surface

membrane. Since *p*-coumaric acid can be incorporated into SE-treated cells (manuscript in preparation), it is reasonable to speculate that such lignin precursor may be involved in the induction of anti-UV activity. We have also found (19) that commercially available alkali-lignin structures show comparable or slightly higher anti-UV activity (SI>61.5) (Table I, Group A). Measurement of the anti-UV activity of bio-lignin may verify the real activity of lignin separated from a botanical extract containing other ingredients.

The relationship between skin protection and quality of life (QOL) is well-recognized. Early diagnosis and sun-protective treatment of erythropoietic protoporphyria (39), non-melanoma skin cancer (40) and neurological, ocular and dermatological abnormalities (41) have been reported to increase the QOL. Therefore, the task to keep health and skin in good condition is influenced by nature's healing ability.

One of our on-going studies is the investigation of SE on the expression of long interspersed nucleotide element-1 (*LINE-1; L1*). About 45% of the human genome is composed of transposable elements. *L1* is the most abundant component of retroelements, comprising about 17% of the human genome of which 80-100 copies are competent as mobile elements (retrotransposition: *L1-RTP*). Although the genetic structures modified during *L1-RTP* activation have been clarified, little is known about the cellular signaling cascades involved. This gene is activated by UV and various chemicals or ligands, such as methamphetamine, cocaine, 6-formylindolo[3,2-b]carbazole (FICZ; produced by UV irradiation from tryptophan), 7,12-dimethylbenz[a]anthracene (DMBA) and 12-*O*-tetradecanoylphorbol 13-acetate (TPA), whereas *SOX2* and *MECP2* are genes that inhibit the function of *L1* by binding to promoter region of *L1* (42) (Figure 3). A preliminary study demonstrated that SE alone did not significantly affect L1 protein expression and we are currently investigating the combination effect of SE and UV on the L1 expression.

Conflicts of Interest

The first author (HS) was supported by Daiwa Biological Research Institute Co., Ltd., Kanagawa, Japan. The Authors wish to confirm that such financial support has not influenced the outcome or the experimental data.

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