IFSCC 2025 full paper (IFSCC2025-1176)

Principal components analysis in various cordyceps extracts and their benefits against sun damaging

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1. Introduction

Cordyceps is a rare naturally occurring entomopathogenic fungus usually found at high altitudes on the Himalayan plateau and a well-known medicinal mushroom in traditional Chinese medicine [1,2]. Many bioactive components have been extracted from cordyceps sinensis including nucleoside, polysaccharide, sterol, protein, amino acid, and polypeptide [3]. The chemical constituents and their corresponding pharmacological actions of cordyceps were reported in the literatures [4,5]. These corresponding pharmacological actions were shown in the studies such as anti-inflammatory, antioxidant, antitumour, antiapoptosis, and immunomodulatory actions [6]. Although there are possible wide range of nutraceutical potential, along with pharmacological actions of cordyceps, limited applications have been reported in cosmetic industry, especially against sun damaging in skin aging.

Skin aging is the most common dermatologic concerns, and the extrinsic skin aging is superimposed on intrinsic skin aging process due primarily to sun damaging (solar ultraviolet radiation) and partly by other factors, such as infrared light, smoking and air pollutants [7]. UV light acts directly on DNA in melanocytes to form cyclobutane pyrimidine (CPD) even hours after UV exposure ended, which if not repaired, subsequently result in a mutation, a cytosine-to-thymine change linked to melanoma, an aggressive type of skin cancer [8]. Thioredoxin-interacting protein (TXNIP) is a small ubiquitously expressed redox active protein that is important for maintaining the reducing milieu of the cell, in part by reducing protein disulfide bonds that occur in response to oxidative processes, which also play an important role during skin aging [9,10].

In this study, cordyceps and cordyceps mycelium were extracted by maceration and PSR™ extraction, and the principal components, such as amino acids, organic acids, vitamins, sugars and proteins, were analyzed by LC-DAD and LC-MS methods. The CPD level of UVB-stressed (200 mJ/cm²), as well as TXNIP level of UVA-stressed (5 J/cm²) normal human ex vivo skin, were detected by immuno-fluorescence and subsequent image quantification. The influence of 0.5% macerate extract, PSR Mycelium and PSR Real cordyceps on these protein expressions were studied and compared.

2. Materials and Methods

Cordyceps and mycelium cordyceps powders were purchased from the market in China, and glycerin and 1,3-propanediol (PDO), from local suppliers, were used to extract the powders.

2.1. Composition analysis

All composition in cordyceps and mycelium cordyceps extracts were separated by liquid chromatography (LC) with different columns and solvent phase. Amino acids were identified and quantified by UV spectrophotometer at 254nm; and protein profile at 280nm. Organic acids, vitamins and sugars were analyzed and quantified by mass spectrometry (MS) with probe temperature at 600°C and source temperature at 120°C.

2.2. Cell viability

All Cordyceps and mycelium cordyceps extracts (0.5% aqueous solution) were applied on Normal human keratinocyte with one application per day, versus untreated as control. After 48h, the cell viability was examed by MTT assay.

2.3. Comet assay

All Cordyceps and mycelium cordyceps extracts (0.5% aqueous solution) were applied on Normal human keratinocytes with one application per day for 2 days, versus untreated as control, and irradiated by UVB at 60 mJ/cm². After 24 hours treatment, the DNA damage level was evaluated by Comet assay and subsequent image quantification.

2.4. CPD evaluation

All Cordyceps and mycelium cordyceps extracts (0.5% aqueous solution) were applied topically on Normal human ex vivo skin with twice a day for 2 days, and irradiated by UVB at 200 mJ/cm², versus vitamin C at 200 µg/ml as positive control and PBS as negative control. After 24 hours treatment, CPD level was evaluated by immuno-fluorescent detection and subsequent image quantification.

2.5. TXNIP evaluation

All Cordyceps and mycelium cordyceps extracts (0.5% aqueous solution) were applied topically on Normal human ex vivo skin with twice a day for 2 days, and irradiated by UVB at 5J/cm², versus vitamin C at 200 µg/ml as positive control and PBS as negative control. After 24 hours treatment, TXNIP level was evaluated by immuno-fluorescent detection and subsequent image quantification.

3. Results

1.1. Composition of cordyceps extracts

The PSR technology was used to extract real cordyceps and cordyceps mycelium. 40% 1,3-propanediol (PDO) with/without 10% glycerin and water were used as solvents. As showed in Table 1, the dry matters are 7.5g/kg and 8.6g/kg, which is much higher than real cordyceps extracted by maceration (0.8g/kg).

Table 1. Physical properties of various cordyceps extracts

	real cordyceps	**cordyceps mycelium**	**real cordyceps**
Solvent	40% PDO	40% PDO/10% glycerin	40% PDO
Technology	PSR™	PSR™	Maceration

Dry matter (g/Kg)	7.5	8.6	0.8
Density (g/cm3)	1.035	1.033	1.029

In the extracts, the conc. of proteins, saccharides, phenolic compounds, organic acids and vitamins are also much higher in PSR Real and Mycelium cordyceps extracts than in macerate extract (Figure 1). Meanwhile, the miRNA conc. of PSR cordyceps extracts was analyzed by Bioanalyzer 2100 (Agilent) with average of 10 ppm, as compare to none in macerate extract.

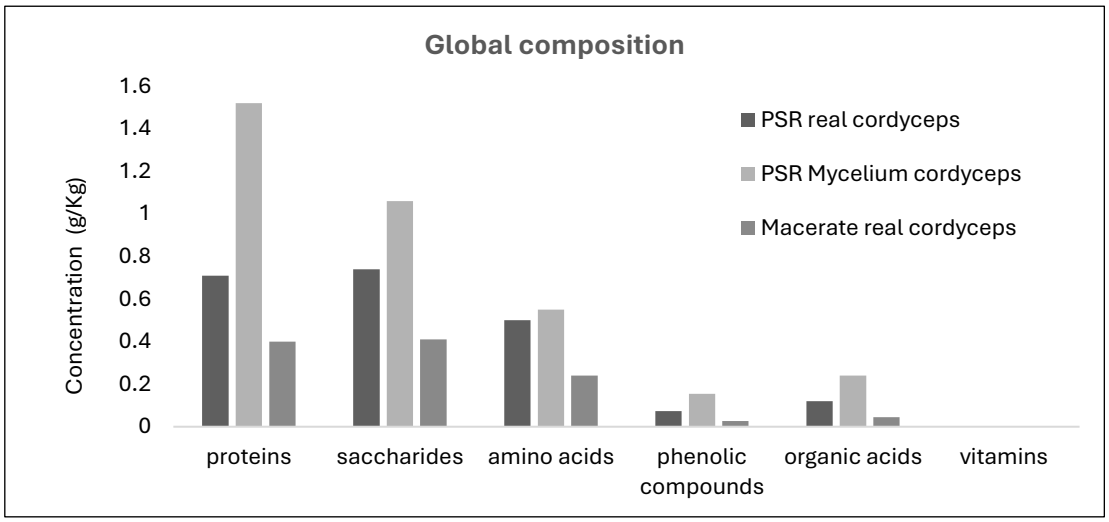

Figure 1. Analysis of principal components in various cordyceps extracts

1.2. Amino acids

PSR real and mycelium cordyceps extracts contain various amino acids, and the concentrations are higher than in macerate extract (Figure 2). Especially, glutamic acid is the main compounds in PSR real cordyceps and proline, alanine and threonine amount are higher in PSR mycelium cordyceps. Among these amino acids, glutamic acid helps to moisturize the skin and keep the skin's pH value balanced. Gly and pro are the two most abundant amino acids in collagen, which play an important role in the production of proline. Alanine is moisturizing agent and play a general role in water retention in the stratum corneum.

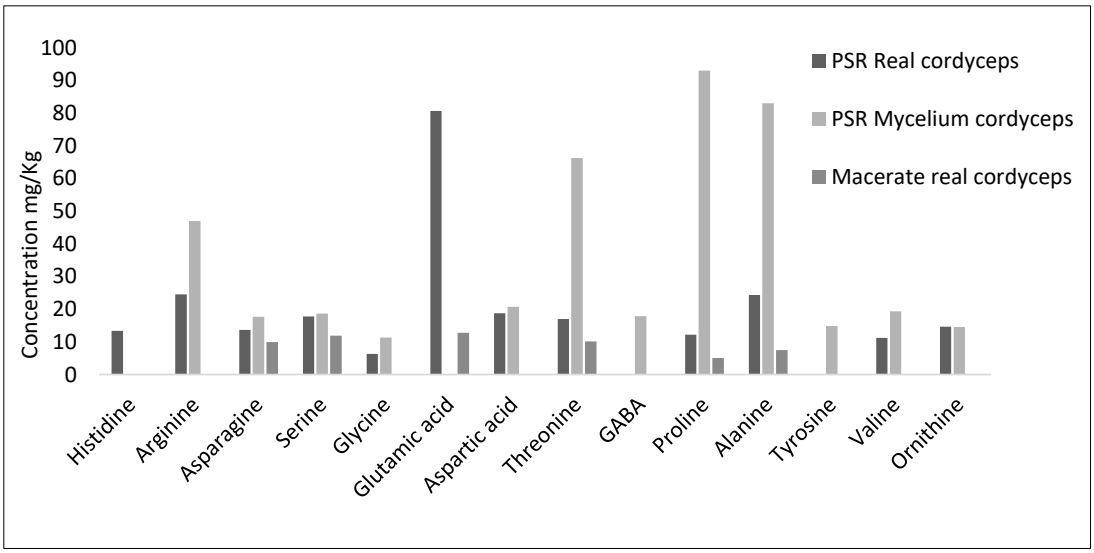

Figure 2. The conc. of amino acids in cordyceps extracts

1.3. Organic acids

PSR cordyceps extracts contain various organic acids and the concentrations are higher than in macerate extract (Figure 3). The main compounds are lactic and malic acids. In skin care, malic acid peels all type of skin (detach and desquam stratum corneum) at the level of 2-10%. It is also used to treat acne, scars, melasma, hyperpigmentation, roughness age spots and seborrhea. Alpha Hydroxy Acids (AHAs) can improve wrinkled skin by increasing the synthesis of glycosaminoglycans and thickening the skin. Lactic acid on skin could increase cell turnover and help eliminate accumulated dead skin cells on the epidermis, reduce the number and depth of fine lines and wrinkles, and improves skin texture and acne. It could also stimulate collagen synthesis and increase epidermal and dermal firmness and thickness.

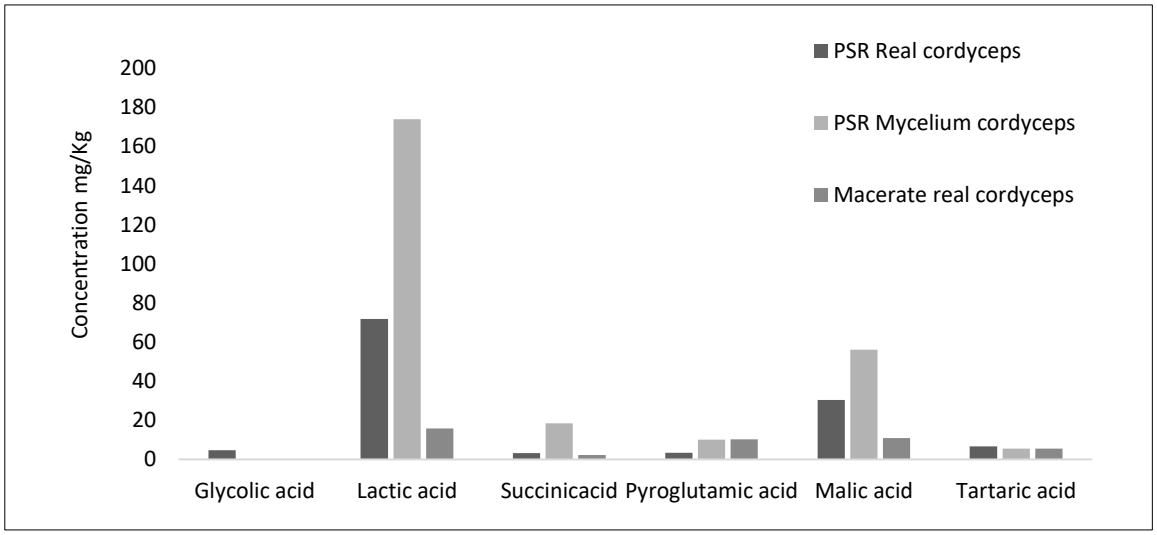

Figure 3. The conc. of organic acids in cordyceps extracts

1.4. Vitamins

PSR real and mycelium cordyceps extracts contain 2 vitamins, vitamins B3 (nicotinamide and nicotinic acid), and the concentrations are higher than in macerate extract. Nicotinic acid also known as niacin, is a form of vitamin B3 (Figure 4). It helps to reduce acne, and reduce wrinkles caused by long-term sun exposures. The recommended dietary allowance is about 16/14 mg per day. Niacinamide is a component of important coenzymes involved in hydrogen transfer. It has a stabilizing effect on epidermal barrier function (at 2%). Niacinamide leads to an increase in protein synthesis (e.g. keratin), has a stimulating effect on ceramide synthesis, speeds up the differentiation of keratinocytes, and raises intracellular NADP levels. In ageing skin niacinamide improves the surface structure (at 2.5%), smoothes out wrinkles (at 5%) and inhibits photocarcinogenesis (500 mg twice a day).

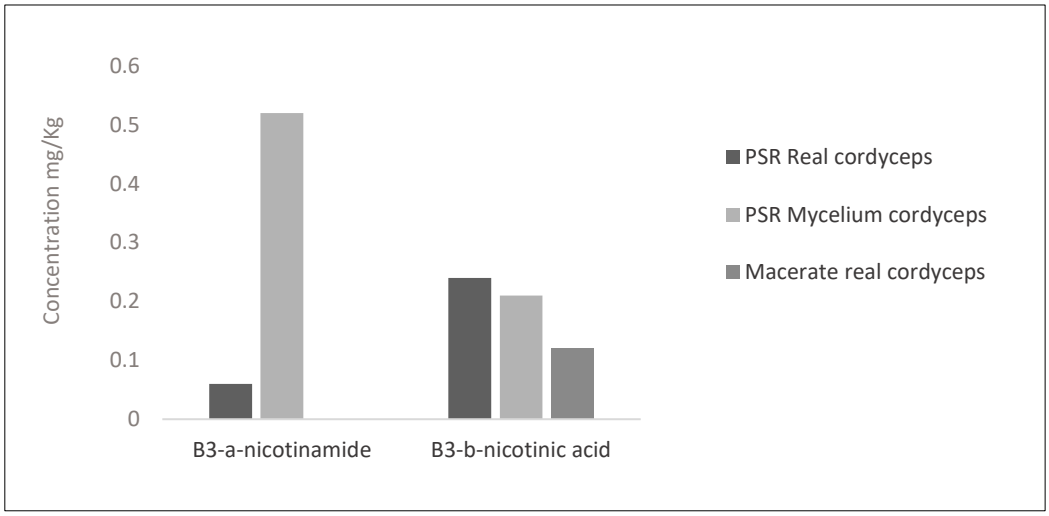

Figure 4. The conc. of vitamins in cordyceps extracts

1.5. Sugars

PSR real and mycelium cordyceps extracts contain various sugars, and the main compound is mannitol (Figure 5). Cordyceps use mannitol as carbohydrate reserve, it plays a role in osmo-regulation and the control of metabolic pathways.

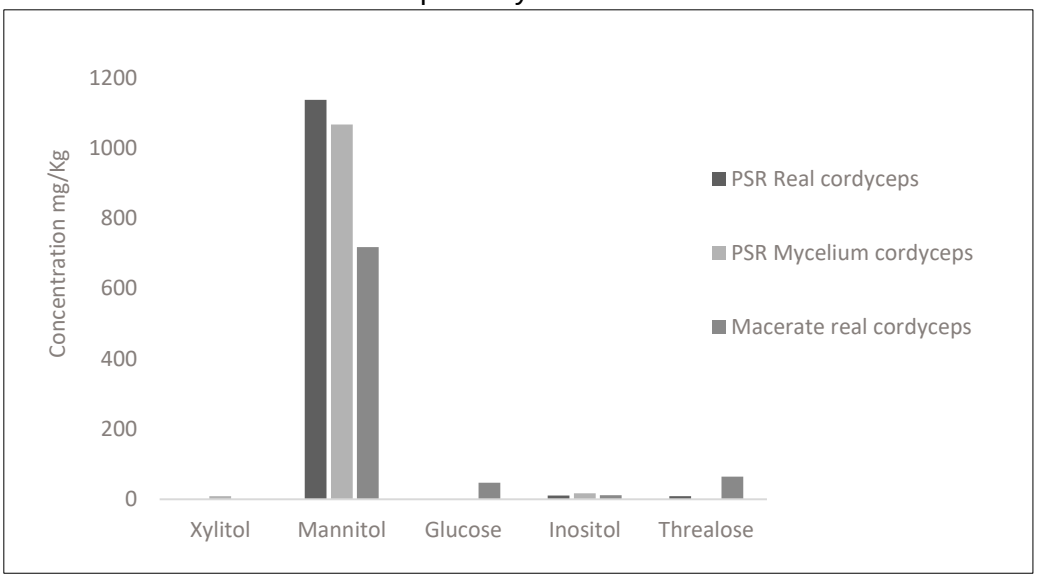

Figure 5. The conc. of sugars in cordyceps extracts

1.6. Protein profile

The protein profiles of cordyceps extracts were evaluated by steric exclusion chromatography. PSR real and mycelium cordyceps extracts show similar protein profiles and Macerate real cordyceps have a much lower distribution size of protein profile as showed in Figure 6.

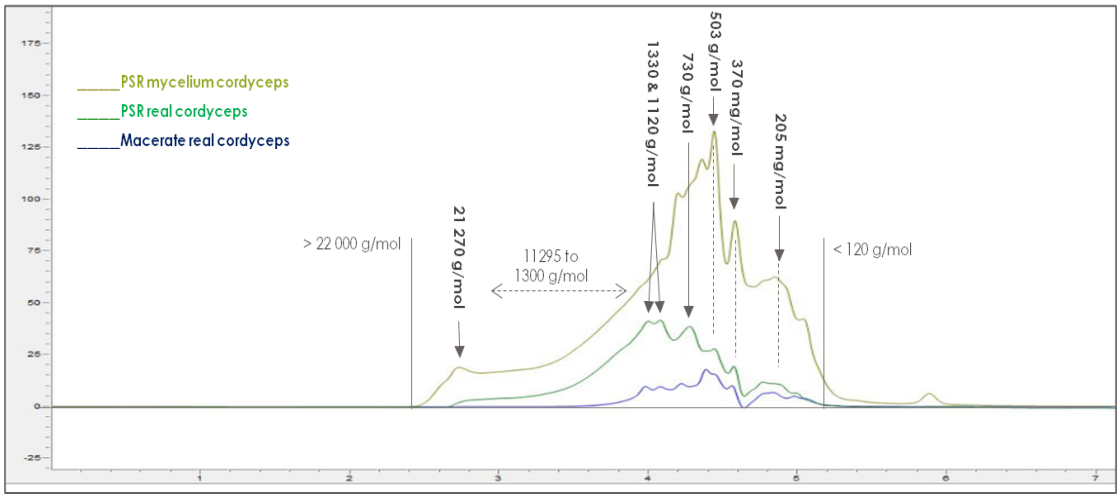

Figure 6. The protein profile of cordyceps extracts

1.7. Cell viability

The cell viability of 0.5% various cordyceps extracts were evaluated on keratinocytes. A 48-hour application of all the extracts did not induce a loss of cell viability which indicate there is no toxicity to the cells after use at 0.5%.

1.8. DNA damage in UVB stressed keratinocytes

Normal human keratinocytes were irradiated by UVB at 60 mJ/cm², and the DNA damage level was evaluated by Comet assay and subsequent image quantification. The UVB-stressed keratinocytes showed DNA damage with all the tested conditions after 24h-treatment, as indicated in Figure 7. The damage levels are less for keratinocytes treated with 0.5% PSR real and mycelium cordyceps extracts.

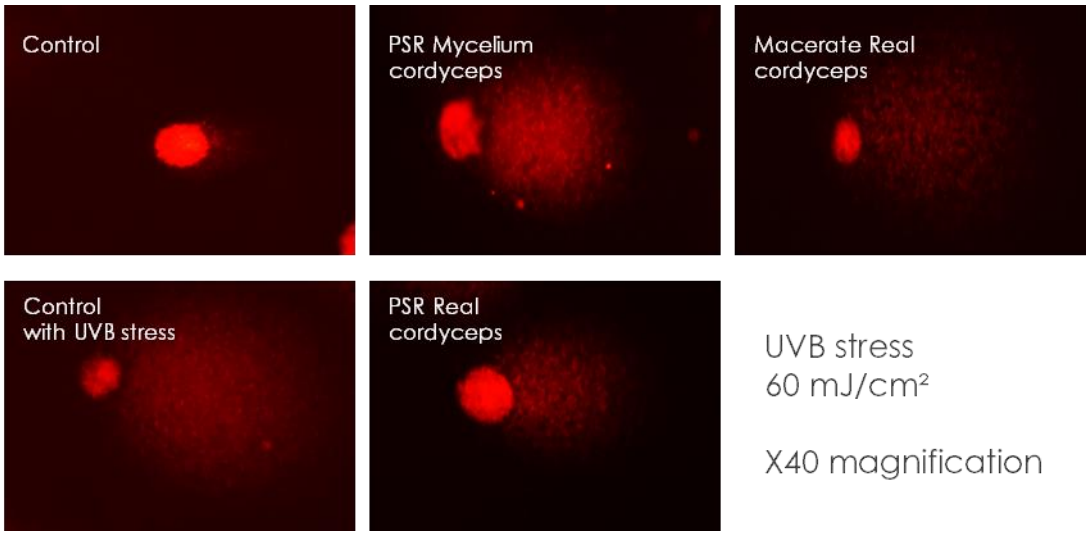

Figure 7. DNA damage level in control and UVB stressed keratinocytes

By subsequent image quantification, the data showed tail moment (%) of DNA damage deceased 24% with 0.5% macerate extract treated sample, while PSR real and mycelium

cordyceps extracts treated samples decreased 35% and 46%, as showed in Figure 8. A 48-hour application of PSR Mycelium cordyceps and PSR Real cordyceps at 0.5% decreased significantly UVB-induced DNA damage in keratinocytes.

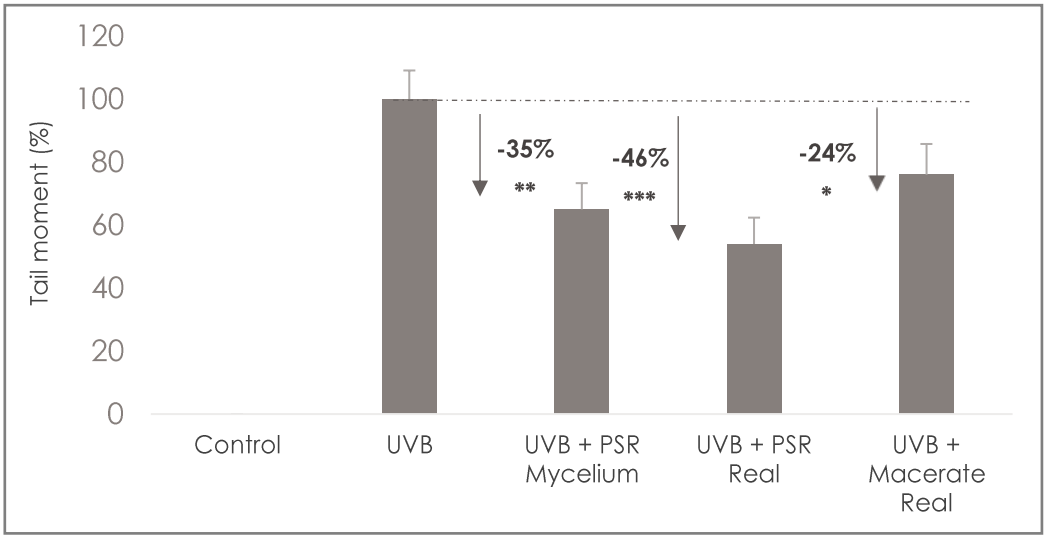

Figure 8. Tail moment (%) of DNA damage in control and UVB stressed keratinocytes, (mean ± sem; n=47), *: significant; **: very significant; ***: highly significant with Wilcoxon test

1.9. CPD level in UVB stressed ex vivo skin

Normal human ex vivo skin was irradiated by 200 mJ/cm² UVB and the CPD level was detected by immuno-fluorescence and subsequent image quantification. As showed in Figure 9, a 48-hour application of 0.5% PSR Mycelium cordyceps and PSR Real cordyceps decreased significantly UVB-increased CPD level in ex vivo skin, with a similar efficacy than positive control, Vitamin C. The macerate decreased CPD expression too, but with lower efficacy than the PSR extracts.

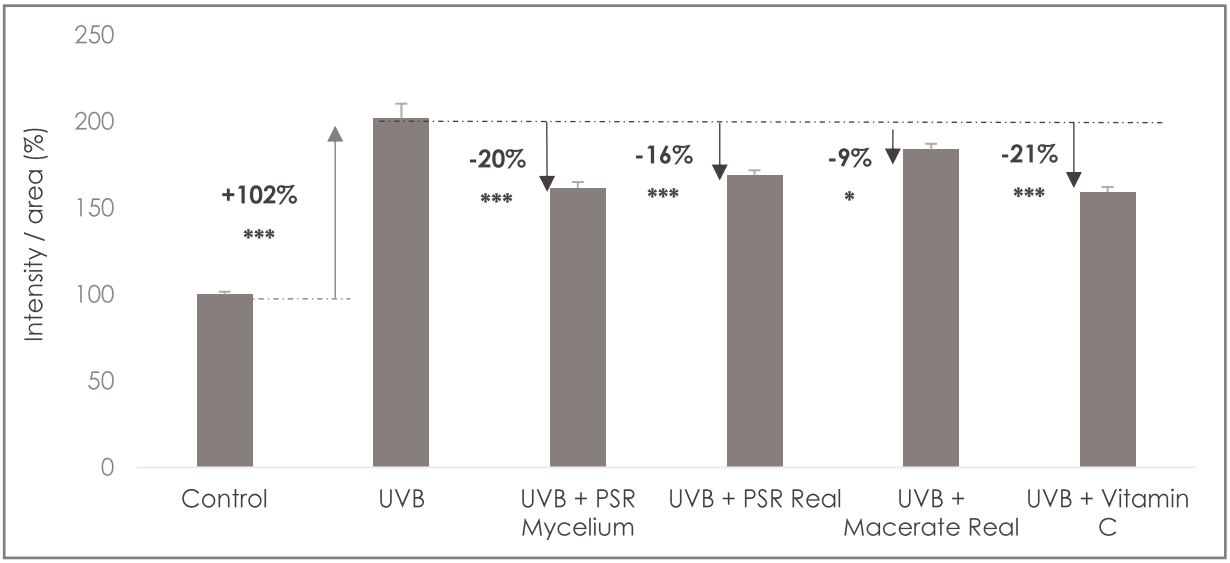

Figure 9. CPD level in UVB stressed ex vivo skin, Unilateral statistical analysis (mean ± sem; n=6), ***: highly significant, *: significant with Student's t-test

1.10. TXNIP level in UVA stressed ex vivo skin

Normal human ex vivo skin was irradiated by 5 J/cm² UVA and the Thioredoxin-interacting protein (TXNIP) level was detected by immuno-fluorescence and subsequent image quantification. A 48-hour application of PSR Mycelium cordyceps and PSR Real cordyceps at 0.5% decreased significantly UVA-increased TXNIP level in ex vivo skin. The vitamin C and the macerate decreased TXNIP expression too but with lower efficacy than the PSR extracts.

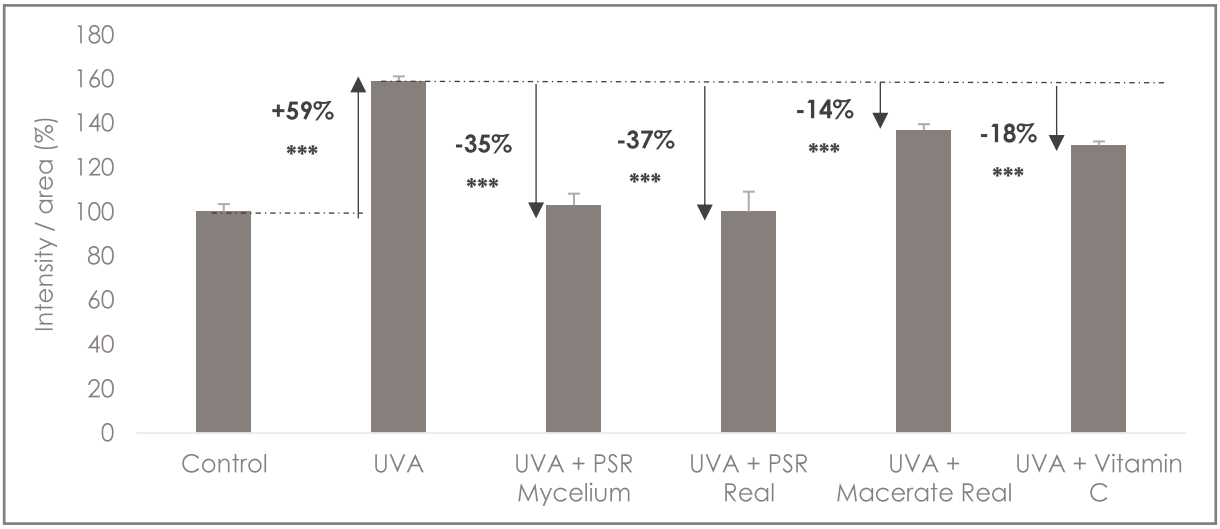

Figure 10. TXNIP level in UVA stressed ex vivo skin, Unilateral statistical analysis (mean ± sem ; n=6), ***: highly significant with Student's t-test

4. Discussion

Skin care products against sun damaging/photoaging has always been a hot topic in cosmetics. Sunscreen products mainly target skin damage caused by UVB (erythema and sunburn), achieving the purpose of anti-aging by absorbing and blocking ultraviolet radiation energy. In recent years, a broader concept was introduced in sun care, for protecting and repairing the damage to mitochondria and elastic fibers caused by infrared radiation and the high-energy part of visible light (blue light radiation). In response to the accumulation of peroxides and free radicals caused by photoaging, as well as the resulting protein carbonylation, glycosylation and lipid peroxidation damage, a large number of brands have conducted basic research and targeted product development on anti-oxidation and anti-glycosylation. Many antioxidants (vitamin C, E and their derivatives, plant flavonoids, polyphenols) and the activation of intrinsic skin antioxidant mechanisms (catalase, superoxide dismutase, coenzyme Q10, longevity factors sirtuins, etc.) have become increasingly common in cosmetic applications over the past few decades. Therefore, how to find more precise and unique mechanisms and targets in the field of combating photoaging has become a brand-new area and research and development direction.

Traditional skin care products against sun damaging are often considered for use during the daytime to more precisely counter immediate sunlight radiation in combination with traditional UV absorbers and physical blockers. However, the products that are usually more expensive and more accepted by consumers are often those used during the night. Based on above knowledge, repairing continuous DNA damage of the Dark Sun effect (cyclobutane pyrimidine dimer, CPDs) and reducing thioredoxin-interacting protein (TXNIP) linked to cell apoptosis and inflammation in skin could serve as brand-new target directions against sun damaging. The

dats showed that mycelium cordyceps and real cordyceps extracted by PSR™ technology could reduce more DNA damage and CPD/TXNIP levels after UV stresses.

5. Conclusion

Mycelium cordyceps and Real cordyceps extracted by PSR™ technology showed higher dry matters and more conc. of principal components. In normal human keratinocytes and ex vivo skin studies, PSR™ extracts could reduce more DNA damage and CPD/TXNIP levels than macerate extract after UV stresses.

Acknowledgments

The authers would like to thank Isabelle Imbert, Audrey Le Mestr and Laurine Bergeron in Ashland Vincience Lab for data preparation and valuable discussion.

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