

APPLICATION OF GREEN TIDE ALGAE *ULVA PROLIFERA* FROM SOUTH YELLOW SEA OF CHINA

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Abstract

Potential utilization of *Ulva prolifera* was studied on water-retention and slow-release material, cosmetics and food. *U. prolifera* was harvested from Rudong sea area. It was indicated that the hygroscopicity of algae powder was 98% of that in glycerin, and the moisturizing of algae ooze reduced to 95% of the initial value after 30 min. *U. prolifera* polysaccharide could alleviate ultraviolet damage to human skin fibroblasts, in which the cells proliferation percentage in experimental group was 32-36% higher than that in negative group. Thus it could be used as additive in skin cream. Furthermore, it was detected that every 100 g *prolifera* powder can provide 17% of energy and 85% of protein required by human body within a day, which has seasoning function in production of peanuts, melon seeds and potato chips. These results provide potential use for *U. prolifera* as water-retention and slow-release material, cosmetics and food.

Key words: *Ulva prolifera*, Hygroscopicity, Moisturizing, Anti-irradiation, Cosmetics, Nutrition, Food processing.

Introduction

Since 2007, Yellow Sea waters of China have broken out a large-scale "green tide" algae disaster for 11 consecutive years (Huo *et al.*, 2013, Liu *et al.*, 2015, Wu *et al.*, 2014). The algae resulting in "green tide", mainly containing *U. prolifera*, will cause serious environmental problems, with the need for timely prevention and treatment (Liu *et al.*, 2013).

On the other hand, *U. Prolifera* can be used as herbal medicine according to Chinese ancient records (Tang *et al.*, 2013). It has abundant healthcare functions including antibiosis (Wei *et al.*, 2015, Xu *et al.*, 2015), anticoagulation (Wang *et al.*, 2013a), antianaphylaxis (Raman *et al.*, 2004), anti-tumor (Hussein *et al.*, 2015), anti-virus (Alberto Aguilar-Briseno *et al.*, 2015), anti-oxidation (Shao *et al.*, 2013, Tang *et al.*, 2013, Wang *et al.*, 2013a, Xu *et al.*, 2015, Zhang *et al.*, 2013), immune regulation (Wei *et al.*, 2014), reduction of blood fat (Teng *et al.*, 2013) and improvement of glucose metabolism (Lin *et al.*, 2015). However, raw material of *U. prolifera* food in current market is mainly acquired through aquaculture, with limited scale. The green tide algae *U. prolifera* will be turned into benefits if it can be used and processed into food.

In this paper, we extracted ooze and polysaccharide from *U. prolifera* to evaluate the hygroscopicity, moisturizing and anti-ultravioletB (UVB) irradiation ability, in order to provide potential use for *U. prolifera* as water-retention and slow-release material and cosmetics. Furthermore, we carried a complete attempt to prepare *U. prolifera* food by scale harvesting and processing, providing reference for development of *U. prolifera* food.

Materials and Methods

U. prolifera was collected from laver culture area in Rudong, Jiangjiasha and Zhugensha, Jiangsu Province. The laver harvesting device consisted of the roller, blade, guardrail and hull (Fig. 1a-c). Before harvesting, we checked the network connection and put net ropes in order. After that, the impurity was removed by artificial screening (Fig. 1d). *U. prolifera* was bagged with seal (about 75 kg each bag). Then, the bags were shipped to the wharf, and landed by the crane. After loading, the goods were transported to the processing plant or laboratory at low temperature.

Human skin fibroblast (HSF) cells from Kunming Cell Bank, Chinese Academy of Medical Sciences were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 15% fetal bovin serum in CO₂ incubator (Thermo Co., U.S.) (Cai *et al.*, 2016, Shin *et al.*, 2014, Vangipuram *et al.*, 2013).

Preparation of algae powder, polysaccharide and ooze:

U. prolifera was completely grinded and sieved to obtain powder. 600g dried frond of *U. prolifera* was pretreated with 75% ethanol, followed by heating and agitating in distilled water at 90°C for 1h. Supernatant was separated using bolting silk and precipitated in final concentration of 75% ethanol followed by centrifugation at 10,000 g under 4 °C for 15 min to acquire polysaccharide. Ooze in bolting silk was centrifuged at 1,000 g under room temperature for 5 min to remove water, followed by vacuum freeze-drying and complete grinding to get algae ooze.



Fig. 1. Harvesting of *U. prolifera* from laver culture area.

Green tide algae were grown in the cable (a), net curtain (b), floating rafts (c). The impurity among algae was removed by artificial screening (d).

Hygroscopicity and moisturizing of algae powder and ooze:

U. prolifera powder and ooze were taken to measure the content of protein, fat, ash, water, and carbohydrate by Kjeldahl method, Soxhlet extraction method (Chatot *et al.*, 1971), 550°C burning method, 105°C dry method at atmosphere, and minusing method, respectively.

The hygroscopicity and moisturizing of powder and ooze was tested according to Wang's method (Wang *et al.*, 2013b) with some modification. In brief, algae powder or ooze was dried to constant weight at 55°C, followed by weighing every 5 min under humidity of 70% at 37°C in hygroscopicity test. Glycerol was used as control and the hygroscopicity percentage was calculated by the following formula: hygroscopicity percentage (%) = $(W2 - W1) / W1 \times 100$, in which W1 and W2 refer to the initial quality and quality after different period, respectively. While in moisturizing test, dried algae powder or ooze was pretreated with deionized water of 10% weight, followed by weighing every 5 min under humidity of 40% at 37°C. The moisturizing percentage was calculated by the formula below:

$$\text{Moisturizing percentage (\%)} = W2 / W1 \times 100.$$

Anti-radiation of polysaccharide: HSF cells were cultured in DMEM medium on 96-well plate with 1×10^4 cells per well for 24h. Then UVB irradiation (UVB radiation meter, Sigma Co.) was added to final dose of 3000-7000 mJ/cm², respectively. Cell viability was determined by MTT method 12 hours later (Roychoudhury *et al.*, 2016).

HSF cells were cultured for 24h in medium as above which was replaced with medium containing

polysaccharide (0.1- 0.5g/L). After another hour, UVB irradiation in final dose of 5000 mJ/cm² was added, and the cellular morphology was observed using Olympus CKX41 inverted phase contrast microscope (Olympus Co., Japan), while cell proliferation rate was determined by MTT method and calculated by the formula below:

$$\text{Cell proliferation percentage} = (\text{Average OD}_{570 \text{ nm}} \text{ value in experiment group} / \text{Average OD}_{570 \text{ nm}} \text{ value in negative control group}) \times 100$$

HSF cells were cultured in routine medium on 96-well plate with 1×10^5 cells per well for 24h, followed by replacement with 0.2 g/L polysaccharide contained DMEM medium. Another hour later, UVB irradiation was added as above. Then the medium was substituted with DMEM containing 10 μM 2, 7-dichlorodihydrofluorescein diacetate (DCFH-DA) for 30 minutes, and distilled water afterwards, for observation using Olympus IX71 fluorescence microscope (Olympus Co., Japan).

***U. prolifera* powder production:** The obtained *U. prolifera* was put in sea water for temporary culture and artificial re-selection. Firstly, *U. prolifera* was washed with sea and fresh water for 3 times, respectively. After 5000 g centrifugal dehydration for 30 min, the frond was diffused into fluffy shape by an algal diffuser. After that, *U. prolifera* was transported to the blow dryer for 20 min drying through a conveyor belt with the length 30 m. The dryer was equipped with 7 temperature control programs, where the temperature decreased after increment. The maximum drying temperature reached 55 °C. At last, the dried *U. prolifera* was smashed into algae powder of 40-80 mesh by the grinder (Fig. 2).



Fig. 2. Production of *U. prolifera* powder
a. Dried *U. prolifera* collection , b *U. prolifera* powder

For *U. prolifera* powder, we measured main nutrients, such as protein, lipid and carbohydrates, and calculated human nutrient reference value (Reid *et al.*, 2013) (i.e., the percentages of total energy and nutrients per 100g food in the requirement of human body for one day). For obtained *U. prolifera* powder, we tested color, taste, mouth feel, impurity, moisture, total number of colonies and *Escherichia coli* according to GB/T 23596-2009, GB 19643-2005, GB 2762-2012 and GB 29921-2013.

Statistical analysis: All data were shown as mean \pm standard deviation (n=3 or 6). Statistical differences between the groups were determined by paired sample *t* test in SPSS statistics, and differences were considered to be statistically significant if $p < 0.05$, or extremely significant if $p < 0.01$.

Results and Discussion

Utilization of *Ulva prolifera* on water-retention and slow-release material

On composition: Carbohydrate in *U. prolifera* powder was greatly lost after alcohol treating and water boiling, and the contents of protein and fat relatively increased. However, the ash content was stable after alcohol washing. In addition, the ooze moisture content after lyophilization was less than that of powder, wherein the latter which contained mucilage was accountable as multifunctional ingredient in terms of moisturizing and thickening agent (Chen & Chen, 2003) (Table 1).

Hygroscopicity and moisturizing of algae powder and ooze: Algae powder and ooze are natural candidate materials for algae mask with service time of 30 min. Therefore, we tested the hygroscopicity of these two materials during the period. The results showed that the hygroscopicity ability of algae ooze was always lower than that of algae powder which might due to the loss of water absorption material, e.g. chitin and its derivatives

(Lin *et al.*, 2012, Sun *et al.*, 2006, Yuan *et al.*, 2008) and polysaccharide (Wang *et al.*, 2013b). Under the same condition, the hygroscopicity ability of *U. prolifera* powder could reach 98% of glycerin in 30 min (Fig. 3). Glycerinum is a common cosmetic additives used as hygroscopicity agent (Bonte, 2011).

Extracts from red algae *Chondrus crispus* and green algae *Codium tomentosum* and *Chlorella vulgaris*, have moisturizing activity (Wang *et al.*, 2015). In this experiment, the results showed that the moisturizing ability of algae ooze is more than 90% after 30 min, which was significantly higher than that of powder, which means the mucilage in power did not work.

With good hygroscopicity and moisturizing ability, the powder and ooze of *U. prolifera* could be used as water-retention and slow-release material, e.g. mask of algae powder and ooze, which can moisturize the skin (Cai *et al.*, 2015a, Cai *et al.*, 2015b) (Fig. 4a,b).

Utilization of *Ulva prolifera* on cosmetics

Polysaccharide preparation: Kinds of procedures were applied on polysaccharide extraction (Alves *et al.*, 2013, Cai *et al.*, 2017). Water-soluble polysaccharide were extracted from *Ulva clathrate* at 60°C through three media (water, EDTA and HCl) with yield of 7.7%, 10.9% and 14.8%, and polysaccharide content of 27%, 30% and 45%, respectively (Hernandez-Garibay *et al.*, 2011), while *Ulva intestinalis* polysaccharide were extracted by ultrasound-assisted response surface methodology with yield of 8.3% and polysaccharide content of 65% (Rahimi *et al.*, 2016). Besides, hot water extraction plus ultrafiltration were applied on *Ulva* sp., which produced polysaccharide with yield of 5% and polysaccharide content of 41% (Adrien *et al.*, 2016). In this paper, crude polysaccharide from *U. prolifera* were prepared with yield of 24.4% and polysaccharide content of 50% according to phenol-sulfate acid methods, which was in line with previous reports that yield of extracted ulvan varied between 1.2 to 27.5% (Alves *et al.*, 2013).

Table 1. Components in powder and ooze of *U. prolifera*.

Mass percentages (%)	Protein	Fat	Moisture	Ash	Carbohydrate
<i>U. prolifera</i> powder	25.85 \pm 1.20	0.17 \pm 0.40	2.23 \pm 0.10	24.32 \pm 0.33	47.43 \pm 1.32
<i>U. prolifera</i> ooze	38.72 \pm 1.30	2.52 \pm 0.60	0.62 \pm 0.30	26.51 \pm 0.57	31.63 \pm 1.10

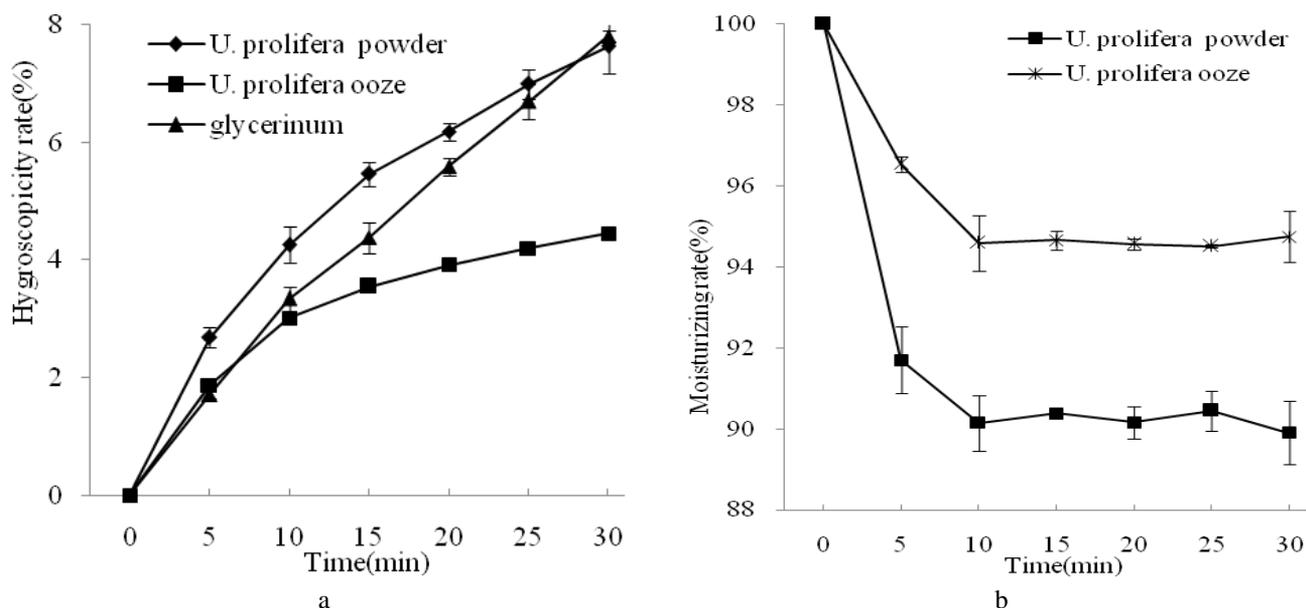


Fig. 3. Hygroscopicity and moisturizing of powder and ooze from *U. prolifera* at 37°C
a. hygroscopicity percentage under humidity of 70%; b. moisturizing percentage under humidity of 40%. n=3



Fig. 4. Model of polysaccharides and cosmetics from *U. prolifera*
a. *U. prolifera* powder derived mask, b. *U. prolifera* ooze derived mask, c. polysaccharides from *U. prolifera*, d. *U. prolifera* polysaccharide derived skin cream, e. *U. prolifera* polysaccharide derived astringent

Inhibition of UVB irradiation induced HSF cell injury: UVB (290-320nm) occupies the largest amount among the three ultraviolet rays reaching earth surface (Reagan-Shaw *et al.*, 2006). UVB causes skin cancer more easily since it is 1,000 times more burning damage to skin than UVA (Cleaver & Crowley, 2002). Inhibition of UVB irradiation by polysaccharide could be tested using induced HSF cell injury model. Differentiated from the mesenchymal stem cell in embryo stage (Bhowmick *et al.*, 2004), HSF cell can composite and secrete collagen,

fibronectin, laminin, hyaluronic acid and other extracellular matrix (Fusco *et al.*, 2007). Collagen and elastin can congregate as collagenous fiber and elastic fiber outside the cell to support skin. As one of the main effective cells in corium layer, HSF cells play an important role in skin aging (Pierard & Pierard-Franchimont, 1997). In the HSF injury model, the cells number gradually decreased as UVB irradiation dose increase, among which the LD₅₀ was 5000 mJ/cm² (Fig. 5). Ulvan from *Ulva* sp. might be of significant interest

for skin care treatments as this ulvan extract significantly promoted hyaluronan biosynthesis by dermal fibroblasts (Adrien *et al.*, 2016). After adding of polysaccharide, the resistance of HSF cells to UVB irradiation induced injury was significantly enhanced, in which the cell survival rates were 132-136% of that in negative control group (Fig. 6). Furthermore, the morphology of most cells in experiment group was adherence growth, greatly different from negative control group (Fig. 7). When injured by UVB irradiation, kinds of reactive oxygen species (ROS), e.g. OH[·], O²⁻ and H₂O₂, in skin cells are generated to oxidize DCFH into DCF which launch green fluorescent (LeBel *et al.*, 1992). The fluorescence intensity in experiment groups was between those of negative control and blank control, which meant polysaccharide could inhibit UVB irradiation induced injury on HSF (Fig. 8). Combining with previous research, we found that *U. prolifera* polysaccharide could protect HSF from being injured by hydrogen peroxide and UVB irradiation (Cai *et al.*, 2016).

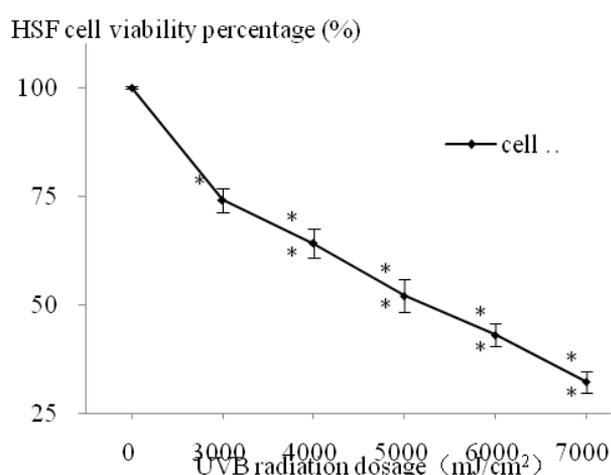


Fig. 5. Influences of UVB on proliferation of HSF. HSF cells were laid on 96-well plate with cells per well followed by irradiation of UVB. Cell viability was determined by MTT. Compare with blank control group (without irradiation of UVB), **means $p < 0.01$. $n = 6$.

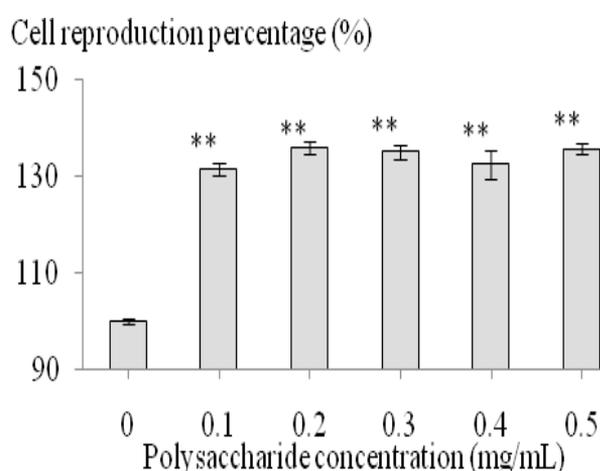


Fig. 6. Protective effects of *U. prolifera* polysaccharide on HSF from UVB irradiation induced injury compare with negative control group, **means $p < 0.01$.

Potential of cosmetics additive of *U. prolifera* polysaccharide: Polysaccharides from green algae, with novel structures and interesting biological activities, have been applied in food, pharmaceutical and medical industries, as well as microbiological and biotechnological applications (Alves *et al.*, 2013). In this study, *U. prolifera* polysaccharide was used for the formulation of cosmetics, just like *Ulva lactuca*, *Sargassum muticum*, chestnut burs and winery byproducts (Balboa *et al.*, 2014). Due to the cosmetics effects, the polysaccharide from *U. prolifera* (Fig. 4c) has been used as additive in skin cream (Fig. 4d,e) (Cai *et al.*, 2015c).

Utilization of *Ulva prolifera* on food

Processing of *U. prolifera* products: The cost of harvesting of fixed *U. prolifera* is lower than that of floating *U. prolifera*. The most-vigorous-growth period of green algae in Rudong area is at the end of seaweed culture. In order to prevent propagation of green tide and turn "waste" into wealth, the experiment used seaweed harvesting device provided by Jiangsu Xianzhuyuan Aquatic Food, Ltd. to harvest the *U. prolifera* on cables followed by processing into *U. prolifera* powder.

U. prolifera is of hollow tubewherein water is difficult to evaporate. Thus chlorophyll damages easily during processing which results in color fading and deterioration. In this experiment, we protected *U. prolifera* from high temperature and direct sunlight during storage and transportation. Then, the *U. prolifera* was performed with centrifugal dewatering after cleaning. It experienced forced air drying in low temperature with 7 drying processes, shortening the drying time. Original green color can be maintained, with simplified production process and lower costs.

In the experiment, 15 tons of *U. prolifera* were processed into about 900 kg *U. prolifera* powders, with moisture content of 4%. The contents of heavy metals, PCBs, harmful colonies, etc. in *U. prolifera* powder were in line with relevant national standards of China. Nutrient analysis showed that as a kind of high-protein food, every 100 g *U. prolifera* powder could provide 17% of the energy and 85% of the protein required by human body per day (Table 2).

Preparation of *U. prolifera* powder flavoring foods: As flavor amino acids reach 2/5 in total amount of amino acids (Wu *et al.*, 2013), *U. prolifera* powder has strong seaweed fragrance. Thus, it is a natural condiment with high price. *U. prolifera* powder is added in production of potato chips, peanuts or melon seeds; the unique flavor and aroma of *U. prolifera* can be emanated out through processes of pretreatment, baking, seasoning and frying, etc.

The potato chips were added with 3-5% of *U. prolifera* powder, a little orange juice and various spices to make *U. prolifera* potato chips (Zhao *et al.*, 2016). In addition, *U. prolifera* powders were also mixed with 2-5% flour and twisted to wrap up peanuts or shelled melon seeds. Series of new products including "*U. prolifera* Peanut Kernel" (Fig. 9a) and "*U. prolifera* Melon Seeds" (Fig. 9b) have been produced in Jiangsu Xianzhuyuan Aquatic Food, Ltd., with sales in the market.

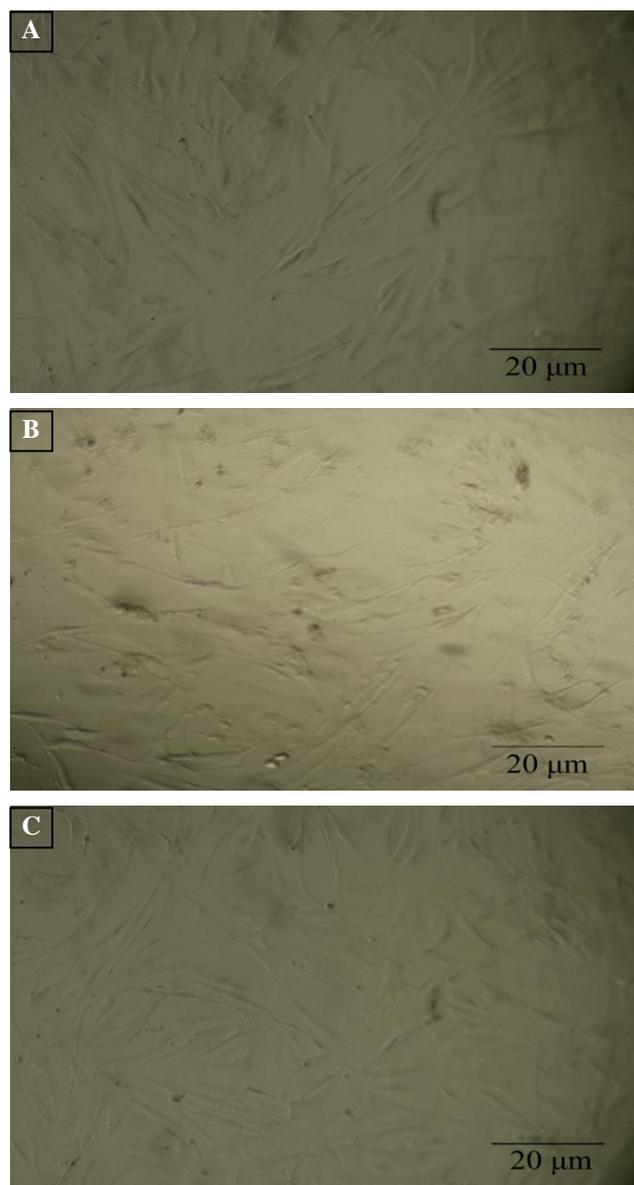


Fig. 7. Morphology of HSF in different treatment groups
a. blank control, b. negative control (5000 mJ/cm² UVB), c. experimental group (5000 mJ/cm² UVB +0.2 mg/mL polysaccharide)

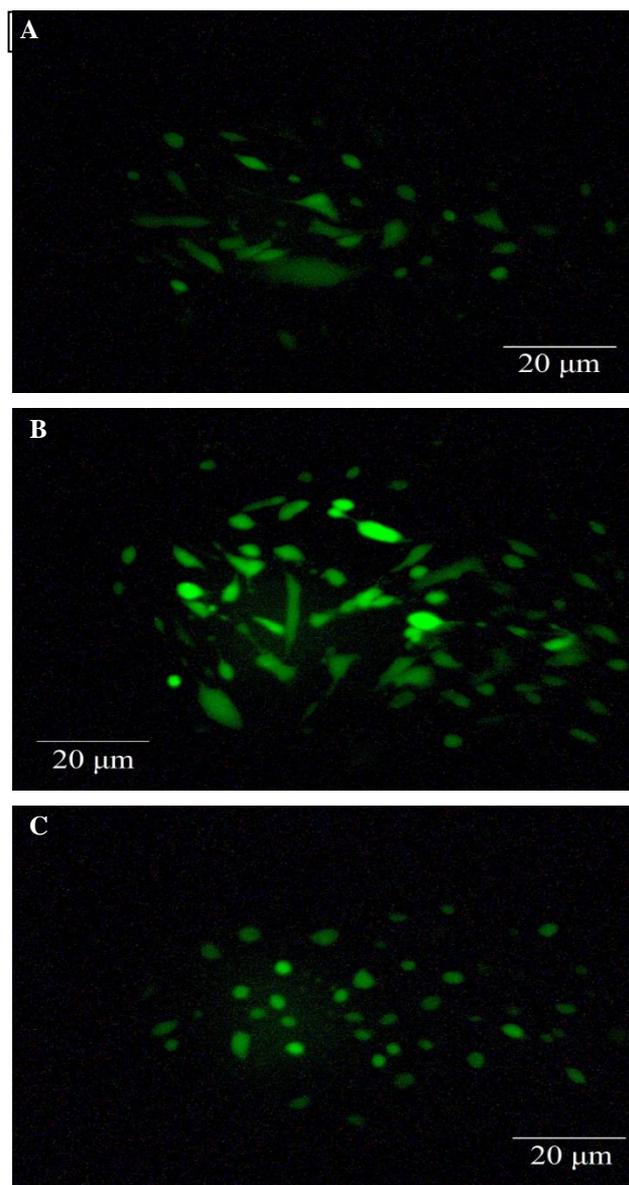


Fig. 8. Cellular ROS assay by DCFH-DA detection
a. blank control, b. negative control (5000 mJ/cm² UVB), c. experimental group (5000 mJ/cm² UVB +0.2 mg/mL polysaccharide)



Fig. 9. *U. proliferata* products after processing, a. *U. proliferata* Melon Seeds, b. *U. proliferata* Peanut Kernel

Table 2. Nutritional information of *U. prolifera* powders.

Nutrient	Content (100g dw)	Nutrient reference values (%)
Energy	1456kJ	17%
Protein	51g	85%
Fat	7g	11%
Carbohydrates	13g	4%
Sodium	471mg	24%

Conclusions

The hygroscopicity of *U. prolifera* powder was similar to that of glycerin, and the moisturizing of ooze reduced to 95% of the initial value after 30 min. *U. prolifera* polysaccharide could alleviate UVB damage to HSF. In addition, *U. prolifera* powder has seasoning function in production of peanuts, melon seeds and potato chips. These results provide potential use for *U. prolifera* as water-retention and slow-release material, cosmetics and food.

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