ACADEMIA JOURNAL OF BIOLOGY 2018, 40(3): 106–112
DOI: 10.15625/2615-9023/v40n3.12686

EVALUATION OF BIOACTIVITIES AND FORMULATION OF FACE MASK FROM *Sargassum* sp. EXTRACT

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ABSTRACT

Brown seaweeds, particularly *Sargassum* genus, contain large amount of polysaccharides, such as alginate and fucoidan, which are widely applied to related health care approaches. Obviously, this genus is a potential alternative resource for cosmetic industry with its extract gained a great deal of biochemical composition and bioactivity. This study showed that the protein, lipid, total carbohydrate, chlorophyll and carotenoid contents of *Sargassum* sp. which collected in Khanh Hoa, Vietnam were 10.45 ± 1.15%, 1.90 ± 0.12%, 50.55 ± 1.05%, 0.25 ± 0.03 mg/g and 0.104 ± 0.001 mg/g of dry weight (DW), respectively. The hot water extract of *Sargassum* sp. showed high free radical scavenging activity (85.82 ± 1.89% at 50 mg/mL), antibacterial activity with the range of minimal inhibitory concentrations (MIC) from 25–100 mg/mL and tyrosinase inhibitory activity (16.19 ± 1.05%, 22.94 ± 1.23%, 104.16 ± 4.15% at concentrations of 50, 100 and 200 μg/mL). The formula of face mask from 10% of *Sargassum* sp. extract gained antioxidant and tyrosinase inhibitory activity with 61.67 ± 0.06% and 29.064 ± 0.06%, respectively. This product was also evaluated for the physical properties included organoleptic (brown color with specific odor), pH, homogeneity, spread ability and irritation test. The bacterial criteria, heavy metal levels of face mask were also recognized to evaluate for safety of human health following Ministry of Health standard.

Keywords: Sargassum, antibacterial, antioxidant, biochemical composition, face mask, extract, tyrosinase inhibitory activity.

Citation: Dang Diem Hong, Phan Bao Vy, Ngo Thi Hoai Thu, Tran Xuan Khoi, Nguyen Cam Ha, Luu Thi Tam, 2018. Evaluation of bioactivities and formulation of face mask from *Sargassum* sp. extract. *Academia Journal of Biology*, 40(3): 106–113. https://doi.org/10.15625/2615-9023/v40n3.12686.

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Received 20 June 2018, accepted 25 September 2018

INTRODUCTION

The cosmetic industry is becoming a key industrial sector with a highly profitable segment, for instance, the market volume of the cosmetic industry in the Europe, United States and Japan is about € 72 billion, € 37,8 billion and € 29,3 billion, respectively, according to a 2005 Publication. This industry is always required new ingredients in order to replace raw materials that have been banned or have become unbelieved by customers (Couteau & Coiffard, 2016). Seaweeds are well known with their wealth of bioactive compounds particularly the polysaccharide, carotenoid, vitamins and thus, provide great biological active resources. For example, compounds isolated from seaweed have demonstrated various biological activities such as antibacterial, anticoagulant, anti-viral and apoptotic activities, potential antioxidant, anti-inflammatory properties (Hong et al., 2011). Presently, a variety of seaweeds have been used extensively in cosmetic applications. Compared to the terrestrial plants, seaweeds are natural resources with a great deal of protein, dietary fiber, total lipid, omega-3 fatty acids, amino acids and vitamins A, B, C and E which are essential for cosmeceutical product devel-

opment (Kim et al., 2008). Research on seaweed with the purpose of developing novel skin-whitening and -care agents from marine sources is of great interest in recent years.

Sargassum sp. is a species of edible brown seaweed (Phaeophyta) which commonly found in Vietnam (Hong et al., 2011). It has been used traditionally in folk medicine for treatment of skin-related disorders (i.e. eczema, scabies and psoriasis), antioxidant, anti-inflammatory, anti-wrinkle, antibacterial, anti-aging, antiphotoaging, moisturizing and whitening (Hong et al., 2011; Thomas & Kim, 2013; Hien et al., 2018). Our previous studies have shown that organic solvent and water extracts of *S. swartzii* possess anti-inflammatory, analgesic, antioxidant, hepatoprotective and hypolipidemic properties (Hong et al., 2011; Hien et al., 2018). Additionally, it has been reported that antioxidants may reduce hyperpigmentation and support skin health (Ma et al., 2011). Thus, for applying to local cosmetic industry, firstly, we evaluated the values of some biochemical components in *Sargassum* sp. Then, the *Sargassum* sp. extract was performed vary bioactivities like antioxidant, antibacterial and tyrosinase inhibition activities in *vitro*. Finally, formulation of cream mask and evaluation of its physiochemical and microbiological characteristics from the *Sargassum* sp. extract was investigated.

MATERIALS AND METHODS

Sargassum sp. was freshly collected from Nha Trang, Khanh Hoa (12°33’28.9”N; 109°17’55.1”E) on March, 2018 that was identified by Tran Mai Duc (Nha Trang Institute of Technology Research and Application, Vietnam Academy of Science and Technology). Seaweed sample was cleaned, rinsed with seawater, then dried under dim light and stored at 2–4°C until analysis.

Biochemical composition analysis

Seaweed sample was washed under running tap water and subsequently oven-dried at 60°C, ground into powder with particle size < 1 mm and stored at room temperature in airtight plastic bags. Protein and lipid contents were analyzed as previously described (Hong et al., 2011). Total carbohydrate content was estimated by the phenol - sulfuric acid method (Foster & Cornella, 1961) with glucose as standard. The contents of chlorophyll and carotenoid were performed following to Lichtenthaler (1987). The contents of lead (Pb), arsenic (As), cadmium (Cd) and mercury (Hg) were analyzed by Atomic Absorption Spectrophotometer (AAS) (Hong et al., 2007).

Preparation of seaweed extract

One kg of fresh *Sargassum* sp. with 70–90 percent water by weight was finely ground and extracted with 2 L of distilled water for 9 hours at 60°C under continuous shaking (150 rpm/min) and then the aqueous extract solution was concentrated under a vacuum in a rotary evaporator at 55–60°C with 75 mbar in 10–12 hours. The obtained aqueous extract was evaluated for its bioactivities.

Antioxidant activity

The DPPH radical scavenging capacity of seaweed extract and cream mask were determined based on the method described by Okawa et al. (2001) with slight modifications. Briefly, a total of 190 µL of DPPH solutions (0.1%) was mixed with 10 µL of various seaweed extract concentrations (5 to 50 mg/mL) or cream mask (5 mg/mL). The reaction mixture was incubated for 20 min at 37°C and triplicate measured the absorbance at 517 nm. Ascorbic acid was used as standard control and evaluated for equivalent inhibition. The free radical scavenging activity was calculated in percentage (%) according to the following formula: % of inhibition = 100 – [(ODs) / (ODc) × 100] within ODs is average optical density of the sample and ODc is average optical density of the control sample (no sample, only DPPH, as 0% scavenging activity). IC_{50} value was determined from the plotted graph of scavenging activity versus the concentration of seaweed extracts, which is defined as the amount of antioxidant necessary to decrease in the initial DPPH radical concentration by 50%.

Antibacterial activity

The antibacterial assays were performed as described previously (Thi et al., 2016) with some types of bacteria, for instance, Gram-positive (*Enterococcus faecalis* ATCC299212, *Staphylococus aureus* ATCC25923, *Bacillus cereus* ATCC 13245), Gram-negative bacteria (*Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enterica* ATCC13076) and yeast (*Candida albicans*

ATCC10231) from the National Institute of Food Control (NIFC), (Vietnam). Stock solutions of *Sargassum* sp. extract (25, 50 and 100 mg/mL) were prepared in DMSO solution and the positive controls were streptomycin and cyclohexamide. The minimal inhibitory concentrations (MIC) were recorded as the lowest concentrations inhibiting bacterial and fungal growth.

Tyrosinase inhibitory activity

Inhibitory of the *Sargassum* sp. extracts and cream mask on tyrosinase activity were determined using tyrosinase inhibitor screening kit (Colorimetric) from BioVision (Milpitas, CA, USA), according to the manufacturer's instructions. Kojic acid (0.75 mM) was used as standard control and evaluated for equivalent inhibition.

Preparation of face mask containing seaweed extract

The basic formulation of cream mask was composed by demineralized water (49.95–64.95%), blanose CMC 7HOF (0.5%), emulsifying wax (7%), propylene glycol (5%), belsil DM 10 (4%), glycerin (1%), talc JA 24R (12%), lunamer 42 (0.5%), preservative agent PE 9010 (0.05%) and supplemented with 5–20% of *Sargassum* sp. extract. The control cream mask was prepared without seaweed extracts.

For determination of bioactivity of the cream mask, we made 4 groups of cream mask formulation. Group 1 (negative control): basic formulation of cream mask; Group 2 (positive control): basic formulation of cream mask plus 0.5% vitamin E; Group 3 (trial 1): basic formulation of cream mask plus 10% of *Sargassum* sp. extract; Group 4 (trial 2): basic formulation of cream mask plus 0.5% vitamin E and 10% of *Sargassum* sp. extract.

Irritation test

Skin irritation test is conducted with rabbits one day after the dorsum is shaved. Cream mask (1 g) is applied on the small area (~ 6 cm^2), and then the treated site is covered with a patch. Patch is removed after 4 hours and then, signs of erythema and edema, and the responses are scored at 1, 24, 48 and 72 hours. Erythema and edema are scored with grades from 0 to 4 depending on the severity. Histopathological examination should be considered to clarify equivocal responses. Depending on the severity and reversibility, skin corrosion and irritation is categorized into 1A, 1B, 1C (corrosive), 2 (irritant, mean scores of 2.3~4.0 for erythema or for edema in at least 2 of 3 tested animals) and 3 (mild irritant, mean scores of 1.5~2.3 for erythema or for edema in at least 2 of 3 tested animals) (Draize et al., 1944).

The irritation test of cream masks was examined by patch test on 5–10 female volunteers with the age varied from 30 to 40 years. The quantity of test cream applied per test patch was 20 mg. The test articles were dispensed onto 8 mm Finn Chambers® on Scanpor® Tape (Dpro Scientific Sdn. Bbd., Malaysia), and the patch was applied to normal skin on the forearm. The patch was removed up to 48 hours after patch application. The treatment sites were assessed for the presence of irritation using a 5 point scale 6 hours after patch removal. The degree of irritation was evaluated by visual scoring according to the following scale with grading defined as: 0 = no reaction; 0.5 = barely perceptible, very weak spotty erythema; 1 = slight erythematic, spotty or diffuse; 2 = moderate erythema; and 3 = intense erythema, infiltration, and possible vesicles (Choi et al., 2013).

Skin moisture analysis

The skin moisturizing effect of the *Sargassum* extracts was measured based on the methods of Dal'Belo et al. (2006). The assay was performed on 5–10 female volunteers within age range of 30 to 40 measuring with Skin detector SG-5E (China). Each individual will follow all process prior to test, for instance, first washed forearm with tap water, second let it at least 30 min at room temperature and then applied cream on 6 cm^2 of skin area with the dose of 10 mg/cm^2 for 15 min. All triplicated measurements were taken on the same period of same day. The moisture level was expressed as percentage of increased hydration rate of treated skin compared to untreated skin.

Evaluation parameters of face mask

The physiochemical characteristics of the test products containing 10% of *Sargassum* sp. extract were analyzed, including organoleptic and homogeneity, pH, heavy metals according to Vietnam standard 2627:1993, 2642–1993 and 2640:2007. The pH of product was tested by pH meter. Microorganisms of the test mask were based on Vietnam standard ACM THA 06 and Vietnam standard ISO22718:2015, ISO22717:2015, ISO18416:2015. The heavy metals were identified following to Vietnam standard ACM THA 05.

Statistical analysis

All experiments were performed at least three times independently. Differences between groups were calculated using a student's t-test. Results were deemed statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Chemical composition

Chemical constituents of *Sargassum* sp. were described in table 1. The rate of protein from our study of *Sargassum* sp. gained 10.45 ± 1.15% of dry weight (DW) which was higher than other previous recordings. According to the report of Di Filippo-Herrera et al. (2018), the variation of protein component was different among species and seasons that reached the minimum in May with 5.25% and maximum in March with 8.4% of DW from *S. horridum*. Following to our present publication, the lipid of *Sargassum* sp. was gained quite low concentration which got only 1.90 ± 0.12% of DW. However, this data was also greater than other publications, for example, *S. oligocystum* with 0.46 ± 0.07% (Muraguri et al., 2016). In the other hand, the total carbohydrates got approximately 50.55% of DW from *Sargassum* sp. in this study. Besides, *S. horridum* varied from 44.15–57.7% (Di Filippo-Herrera et al., 2018). In term of carotenoid content, *Sargassum* sp. consisted of 0.104 ± 0.001 mg/g of DW that was not different significantly than *S. vulgare* (0.111 ± 0.03 mg/g of DW) (Ozgun & Turan, 2015). In another constituent of pigment, total chlorophyll, it reached 0.25 ± 0.03 mg/g of DW from *Sargassum* sp. that was approximately triple less than *S. ilicifolium* (0.71 ± 0.02 of DW) and *S. polycystum* (0.77 ± 0.02 of DW) (Nazni & Renuga, 2015).

Table 1. Chemical components of *Sargassum* sp.

Protein (% DW)	**Lipid** (% DW)	**Total carbohydrate** (% DW)	**Carotenoid** (mg/g DW)	**Total chlorophyll** (mg/g DW)
10.45 ± 1.15	1.90 ± 0.12	50.55 ± 1.05	0.104 ± 0.001	0.25 ± 0.03

So, the effect of seasonal variation and geographical location take an important role on the chemical composition. Furthermore, extracted solvent and procedure are also factors caused differences in values of seaweed constituents. Besides, the lead, cadmium and arsenic level in *Sargassum* sp. extract were 0.522, 0.068 and 1.925 ppm, respectively and there was not also presence of mercury that reached within range of tolerable value of quality criteria for safety regulations of cosmetic products.

Bioactivity of *Saragssum* sp. extract

Antioxidant activity of Sargassum sp. *extract*

Table 2 showed the result of percentage of DPPH free radical scavenging activity of *Sargassum* sp. extract with 85.82 ± 1.89% at 50 mg/mL concentration. Our obtained result was higher than *S. baccularia* (45.9 ± 0.427%) and *S. binderi* (44.1 ± 0.224%) at the same tested concentration in the report of Sarini et al. (2014). So, at 50 mg/mL concentration, *Sargassum* sp. extract is considered as better antioxidant agent.

Antibacterial activity of Sargassum sp. *extract*

As shown in table 2, the MIC value of *Sargassum* sp. extract was ranged from 25 to 100 mg/mL. The *Sargassum* sp. extract was the most effective against *S. aureus* (MIC: 25 mg/mL), *E. faecalis* (MIC: 50 mg/mL) while it inhibited *B. cereus, P. aeruginosa* and *S. enterica, E. coli* and *C. albicans* growths at MIC of 100 mg/mL. Compared to the result of evaluating antibacterial activity by determining MIC from Tajbakhsh et al (2011), the hot water extract of *S. oligocystum* was considered as the antibacterial agent for *S. aureus, P. aeruginosa* and *E. coli* with MIC values 3.175 ± 0.064, 3.175 ± 0.000 and 9.556 ± 0.251 mg/mL, respectively. The difference in our results with Tajbakhsh et al (2011) may due to differing strain and extraction conditions.

Tyrosinase inhibitory activity of *Sargassum* sp. extract

The skin whitening ability was assessed by inhibition of tyrosinase for rate-limiting of melanin. Thus, the criteria that tyrosinase inhibitory

is becoming essential and useful for cosmetic products. In this study, the tyrosinase inhibitory activities of *Sargassum* sp. extract at concentrations of 50, 100 and 200 µg/mL were 16.19 ± 1.05%, 22.94 ± 1.23%, 104.16 ± 4.15%, respectively (Table 2). Our obtained result was showed relatively higher or similar with tyrosinase inhibitory activity of the *S. horneri* extract (11.45 ± 0.98%), *S. thunbergii* extract (23.14 ± 1.02%), and *S. coreanum* extract (31.26 ± 0.99%) at concentration of 100 µg/mL (Cha et al., 2011). It can be concluded that the *Sargassum* sp. extract showed potential tyrosinase inhibitory activity as ingredients for cosmetic products.

Table 2. Evaluation of bioactivity from *Sargassum* sp. extract

Antioxidant activity	**Scavenging activity (%)**
Sargassum sp. (50 mg/mL)	85.82 ± 1.89
Antibacterial activity	**MIC (mg/mL)**
Enterococcus faecalis ATCC299212	50
Staphylococcus aureus ATCC25923	25
Bacillus cereus ATCC13245	100
Escherichia coli ATCC25922	100
Pseudomonas aeruginosa ATCC27853	100
Salmonella enterica ATCC13076	100
Candida albicans ATCC10231	100
Tyrosinase inhibitory activity	**% Relative inhibition**
Kojic acid	60.48 ± 2.41
Sargassum sp. (50 µg/mL)	16.19 ± 1.05
Sargassum sp. (100 µg/mL)	22.94 ± 1.23
Sargassum sp. (200 µg/mL)	104.16 ± 4.15

Optimal concentration of *Sargassum* sp. extract in face mask

Seaweed extracts is often found in ingredients of cosmetic packages, particularly in moisturizing products as face, hand and body lotion and creams. Depend on type of skin-care production, difference of concentrations of extract is added in the cosmetic products. For example, 1–20% plant extract usually add to cream products (Choi et al., 2013). In this study, the moisturizing property of the test face mask containing 5, 10, 15 and 20% of *Sargassum* sp. extract was performed. As shown in figure 1, the moisture level of skin of volunteers at concentration of 5, 10, 15 and 20% of *Sargassum* sp. extract increased significantly, approximately 1.5, 1.75, 1.25 and 1.24 fold compared to control sample without seaweed extract. So, the formula with 10% of *Sargassum* sp. extract was considered as optimal concentration for cream mask.

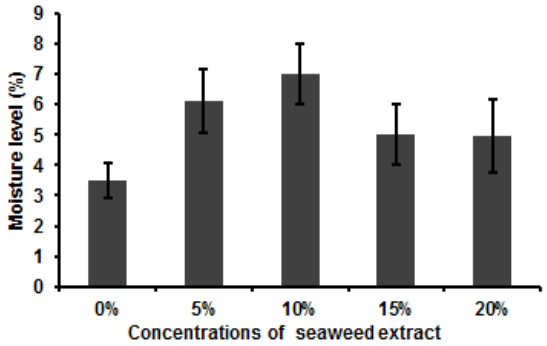

Figure 1. Moisture level of skin after applying face mask with different concentrations of *Sargassum* sp. extracts

Evaluation parameters of cream mask from Sargassum sp. extract

In the purpose of screening a suitable formula of face mask from *Sargassum* sp. extract, some bioassay for estimating moisturizing, antioxidant and tyrosinase inhibitory activity. As shown in figure 2, the moisture level of skin of trial 1 and 2 was more than 1.21 and 1.14 fold compared to negative control. Moreover, antioxidant activity of trial 1 and 2 was 61.67 ± 0.06% and 57.44 ± 0.01% as well as tyrosinase inhibitory activity of the formula 1 and 2 was 29.064 ± 0.060% and 27.07 ± 1.05% (table 3). Hence, the formulation of trial 1 was chosen for evaluating safety criteria.

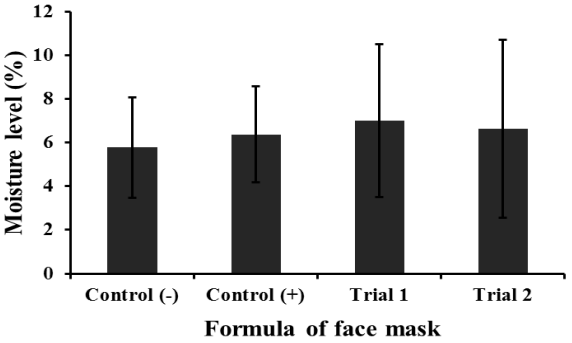

Figure 2. Moisture level of different formula of face mask Control (-): blank formula; Control (+): blank + 0.5% vitamin E; Trial 1: blank + *Sargassum* sp. extract; Trial 2: blank + *Sargassum* sp. extract + 0.5% vitamin E

Table 3. Antioxidant and anti-tyrosinase activity of face mask with *Sargassum* sp. extract

Parameters	Trial 1	Trial 2
Antioxidant activity	Scavenging activity (%)	
	61.67 ± 0.06	57.44 ± 0.01
Tyrosinase inhibitor activity	Inhibitory activity (%)	
	29.064 ± 0.06	27.07 ± 1.05

As shown in table 4, the color of face mask was brown with specific odor with low pH (5.6). The observation of the physical stability at room and cold temperature during 8 weeks of storage indicated that this formula was stable and not separated. Irritation test of cream mask was performed in both of *in vivo* and human trial. Both of models showed that no skin irritation. The spread area of 22.89 cm^2 described semisolid texture and the drying time was quite slow (20 min) because of high amount of water in the formula. Specified microorganisms that were recognized as a skin pathogen species and been harmful for human health are total viable count, *S. aureus*, *P. aeruginosa*, *C. albicans* and yeast. In this study, no specified microorganisms were over-limited in face mask (Table 4). Additionally, there was no the heavy metal elements were found in face mask.

Table 4. Evaluation safety criteria of face mask from aqueous extract

Parameters	Unit	Requiment	Result
Physical properties			
Organoleptic	no	Specific color and odor	Brown with specific odor
Homogeneity	no	Homogen	Homogen
Irritation	no	No significantly or non irritate	Non irritate
Drying time	minute		20
Spreadability	cm^2		22.89
pH		5.00 – 6.00	5.6
Presservative agent	%	< 0.5% (Annex VI)	< 0.5%
Indicator bacteria			
Total viable count	CFU/g	< 100	accepted
Pseudomonas aeruginosa	CFU	No bacteria in 0.1g	accepted
Staphylococcus aureus	CFU	No bacteria in 0.1g	accepted
*Candida albican*s	CFU	No bacteria in 0.1g	accepted
Yeast	CFU/g	< 10	accepted
Heavy metal			
Asen	ppm	≤ 5	accepted
Lead	ppm	≤ 20	accepted
Mercury	ppm	≤ 1	accepted

CONCLUSION

Sargassum sp. extract was used widely in skincare products due to great deal of additional benefits given by contemporary cosmetic researchers. *Sargassum* sp. extract was chosen for antioxidant, antibacterial and tyrosinase inhibitory activity that considered as active ingredient in cosmetic products. Our present results confirmed strongly that face mask with 10% concentration of *Sargassum* sp. extract can be also a potential source of natural bioactivity for maintaining oxidative stability as well as active components of skin care products. Some organoleptic parameters assumed by volunteers that the color of face mask was brown with specific odor and no skin irritation, the drying time was 20 min as well as ability of spreading of product on the skin was 22.89 cm^2. Especially, this product aimed to improve the moisture level of skin as well as possess antioxidant activity with 61.67 ± 0.06% and tyrosinase inhibitory activity with 29.064 ± 0.06%.

Acknowledgements: This study was supported by a VAST 04.05/17-18 project "Study on crude extract from several species of Vietnam seaweed (macroalgae) for cosmetic application" (2017–2018) for Dr. Ngo Thi Hoai Thu.

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