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Screening of Xerosis Inhibitor from Seaweed Extracts Using HaCaT Keratinocyte

Seung-Je Yoon[1], Mohammed N. A. Khan[1], Ji-Young Kang[1], Ju-Hyun Nam[2], Dong-Hyun Ahn[3] and Yong-Ki Hong[1]*

[1]Department of Biotechnology, Pukyong National University, Namku, Busan 608-737, Korea
[2]Division of Food Nutrition and Culinary Arts, Daegu Technical College, Dalseogu, Daegu 704-721, Korea
[3]Department of Food Science and Technology, Pukyong National University, Namku, Busan 608-737, Korea

Abstract The primary function of the skin is to protect the body from the unwanted environmental influences. The outermost layer of the skin is stratum corneum which consists of corneocytes surrounded by lipid regions. Ceramides covalently bound to keratinocytes are essential for the barrier function of the skin, which can be disturbed in the disease, like xerosis. Xerosis is an abnormal dryness of the skin which reduced the thickness of stratum corneum and ceramide content decreasing with age. In this study, 36 seaweed extracts have been tested for screening of xerosis inhibitory agent by *in vitro* HaCaT keratinocyte assay. *Ishige sinicola* and *Helminthocladia australis* induced the significant amount of ceramide-like substance I in HaCaT keratinocyte among the tested seaweed extracts. *Sargassum fulvellum, Chondrus ecellatus* and *Gigartina tenella* also induced the ceramide-like substance I whereas *Helminthocladia australis* and *Pachymeniopsis elliptica* induced the ceramide-like II from HaCaT keratinocyte.

Key words : Ceramide, HaCaT Keratinocyte, xerosis

Introduction

Isolation of nutraceutical compounds from marine algae has been the most talked issue to the scientific investigators. Xerosis, extensively known as dry skin, which referred to roughness, flaky, or scaly skin that is less flexible than normal and dry to feel, is relatively common problem in all age groups, but is more common in elderly individuals [1,2]. The water content of the stratum corneum is of paramount importance in maintaining the normal appearance and textural of human skin. Ceramide 1 is the main repository of stratum corneum linoleic acid, and changes in the levels of ceramide 1 linoleate are associated with cutaneous abnormalities [3].

Intercellular lipids, consisting of ceramides, cholesterol and free fatty acids together with esterified long-chain fatty acid, play an important part in regulating skin barrier homeostasis. Both the qualitative and quantitative compositions of the barrier lipids and, to a minor extent, of the skin surface lipids, are important to maintain an efficient, functioning skin barrier [4,5]. Abundant findings in the literature point to a special role for ceramides in skin barrier functions [6-9]. Consequently, the possibility that ceramide-containing dermatological and cosmetic products could play an important inhibitor of the xerosis.

As terrestrial resources become over explored, attention has turned to the marine environment as an alternative source of drug development. Modern technologies have opened vast areas of research for the extraction of biomedical compounds from oceans and seas. In recent years, many bioactive compounds have been extracted from various marine resources [8] but marine algae have received comparatively less bioassay attention although there are a number of seaweeds with economic potential [11]. The ancient tradition and everyday habit of Asian people have made possible a large number of epidemiological researches showing the health benefits linked to seaweed consumption [12-14]. It will be of great if these species could be the major role players in the development of xerosis inhibitory drugs for human skin. The present work investigated the potential seaweed species that could be used as the xerosis inhibitory agents for the skin aliment

* Corresponding author
Phone: +82-51-629-5862, Fax:+82-51-629-5863
E-mail: ykhong@pknu.ac.kr

as preliminary screening among the species available in the Korean coast.

Materials and Methods

Seaweed extracts

The brown seaweed, *Undaria pinnatifida* f. *distans* (Harvey) Suringar (northern forma), was collected from Kijang aquaculture farm (Busan, Korea) in May 2004 and 2005. Other seaweed species, for activity comparative purposes, were collected from the coast of Korea between October 2003 and June 2005. Voucher specimens have been deposited in the author's laboratory (Y. K. Hong). For each 20 g seaweed powder, 1 l of 100 % methanol was used to extract the methanol-soluble fraction at room temperature for 1 day. To remove salt from the seaweed extracts, methanol extraction was repeated several times (from the previous methanol-soluble fraction) until, to the eye, the amount of salt were negligible. In case of *U. pinnatifida* powder (1 kg), it gave a dark brown residue (11.2 g)-a yield of 1.1 % (Figure1).

Cell culture

The spontaneously immortalized human keratinocyte cell line HaCaT was cultured in Dulbecco's modified Eagle medium (DMEM) with 10 % fetal bovine serum and 100 units/ml penicillin/streptomycin at 37^{0}C in an incubator containing 10% CO_2 [15].

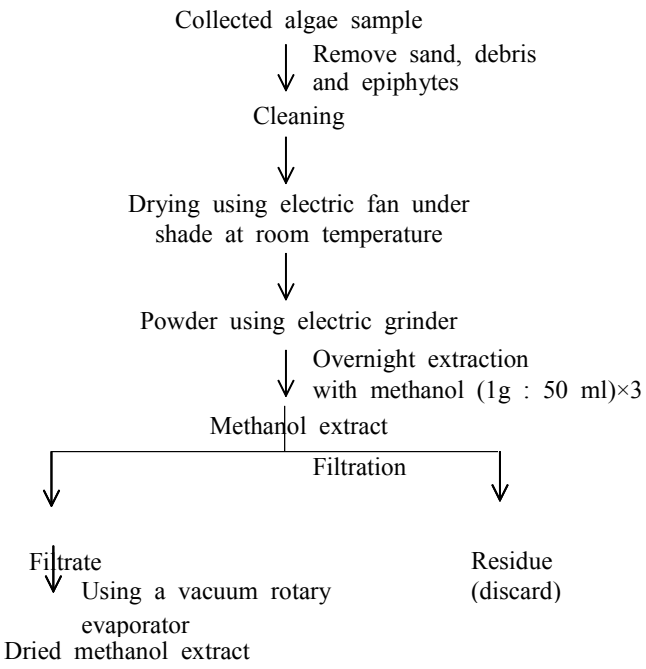

Fig. 1. Experimental protocol of preparing crude algal extracts for preliminary screening.

Lipid extraction from HaCaT

HaCaT keratinocyte was seeded in 12-multiwell culture plate and grown up to approximately 50 % confluence. Then the cells were treated with the seaweed extracts. After incubation for 24h, the cells were washed twice with phosphate-buffered saline (PBS) and were harvested by scraping in 0.88% KCl. One mL of solution (chloroform: methanol: water 2:4:1.6) was used to extract the epidermal lipids from the harvested keratinocyte overnight at -20^{0}C [16]. A mixture of chloroform and water (1:1) was added to the sample and placed in a shaker for 10 min at room temperature. The mixture was then centrifuged at 900 G for 10 min and collected the chloroform fraction for lipid extraction.

TLC and lipid visualization

Thin layer chromatogram was developed twice with chloroform: ethanol: acetic acid (190:9:1) to resolve lipids in the sample. After developing the solvent, the plate was air dried, sprayed with a solution (10 % $CuSO_4$, 8 % H_3PO_4) and charred at 180^{0}C.

Results and Discussion

The present work was carried out with 37 seaweed extracts as preliminary screening of xerosis inhibitor through *In-vitro* HaCaT keratinocyte. The *Ishige sinicola* and *Helminthocladia australis* induced the significant amount of ceramide-like substance I from HaCaT keratinocyte among the tested seaweed species. *Sargassum fulvellum, Chondrus ecellatus* and *Gigartina tenella* also induced the ceramide-like substance I whereas *Helminthocladia australis* and *Pachymeniopsis elliptica* induced the ceramide-like II from HaCaT keratinocyte of the tested all available seaweed species (Table 1).

The stratum corneum as the outermost layer of the skin has a barrier function and protects the organism against environmental influences and transepidermal water loss. Its unique morphology consists of keratin-enriched corneocytes embedded in a distinctive mixture of lipids that contains mainly ceramides, free fatty acids, and cholesterol [17]. Of the three key lipids, ceramides comprise a family of at least seven sub-fractions, which account for up to 50 % of stratum corneum lipids by weight [18,19]. Ceramides are viewed as critical for barrier function, not only because of their quantitative significance, but also because of their amphiphilic structure and extremely long-chain, constituent *N*-acyl fatty acids. Studies with different inhibitors of ceramide formation demonstrate a broad requirement for the ceramide family in barrier func-

Table 1. Effect of seaweed extracts on ceramide production from HaCaT keratinocyte. The cells were grown up to 50 % confluence, and treated with methanol extracts. Ceramide was estimated by TLC after 24 h exposure in DMEM

Seaweed species	Ceramide-like substance I (R_f 0.49)		Ceramide-like substance II (R_f 0.42)		Constitutive (R_f 0.38)	
	1st	2nd	1st	2nd	1st	2nd
CHLOROPHYTA						
Codium fragile	-	+	-	-	+	+
Enteromorpha compressa	-	-	-	-	+	+
Enteromorpha linza	-	-	-	-	+	+
Scytosiphon lomentaria	-	-	-	-	+	+
Ulva pertusa	-	-	-	-	+	+
PHAEOPHYTA						
Colpomenia bullosa	-	-	-	-	+	+
Colpomenia sinuosa	-	-	-	-	+	+
Costaria costata	+	-	-	-	+	+
Dictyota dichotoma	-	-	-	-	+	+
Ecklonia cava	-	-	-	-	+	+
Ecklonia stolonifera	-	-	-	-	+	+
Hizikia fusiformis	-	-	-	-	+	+
Ishige okamurae	-	-	-	-	+	+
Ishige sinicola	++	+	-	-	+	+
Laminaria japonica	+	-	-	-	+	+
Sargassum confusum	+	-	-	-	+	+
Sargassum fulvellum	+	-	-	-	+	+
Sargassum horneri	-	-	-	-	+	+
Sargassum ringgoldianum	-	-	-	-	+	+
Sargassum sagamianum	-	-	-	-	+	+
Sargassum thunbergii	-	-	-	-	+	+
Undaria pinnatifida	-	-	-	-	+	+
RHODOPHYTA						
Carpopeltis cornea	-	-	-	-	+	+
Chondrus ocellatus	++	-	-	-	+	+
Corallina pilulifera	-	-	-	-	+	+
Gigartina tenella	++	-	-	-	+	+
Gracilaria verrucosa	-	-	-	-	+	+
Gymnogongrus flabelliformis	-	-	-	-	+	+
Hypnea charoides	-	-	-	-	+	+
Helminthocladia australis	++	+	+	-	+	+
Lomentaria catenata	-	+	-	-	+	+
Meristotheca papulosa	-	-	-	-	+	+
Pachymeniopsis elliptica	+	ND*	+	ND*	+	ND*
Pachymeniopsis lanceolata	-	-	-	-	+	+
Porphyra yezoensis	-	-	-	-	+	+
Symphyocladia latiuscula	-	-	-	-	+	+

ND* :not determined because of no cell growth.

tion [20]. The functions and the requirements of specific types within the ceramide family are not still completely understood [21].

Many seaweed species are known to have diverse poly-unsaturated fatty acids (PUFAs) [22,23]. Lipid and lip-id-based creams and ointments are most often used for the treatment of eczema, aimed at remedying the reduced lipid content and restoring skin barrier function [24]. Therefore, seaweed could be the potential source for the invention of xerosis inhibitory drugs near future if proper attention has given in this unexplored area. Work is in progress to measure the type and amount of ceramide available in the potential seaweed species.

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