Vietnam Journal of Marine Science and Technology 2022, 22(4) 415–422

Vietnam Academy of Science and Technology

Vietnam Journal of Marine Science and Technology

journal homepage: vjs.ac.vn/index.php/jmst

Screening of algae collected from Nha Trang bay, Vietnam, for potential cosmeceutical bioproducts

Hoang Kim Chi[1], Tran Thi Hong Ha[1], Truong Ngoc Hung[1], Tran Thi Nhu Hang[1], Tran Thi Hong Tham[2,3], Le Mai Huong[1], Le Huu Cuong[1,*]

[1]*Institute of Natural Products Chemistry, VAST, Vietnam*
[2]*VNU University of Science, Hanoi, Vietnam*
[3]*Vietnam-Poland High School, Hanoi, Vietnam*

Received: 12 April 2022; Accepted: 26 August 2022

ABSTRACT

As marine seaweeds are a great source of natural products, investigating biological properties for appropriate management and exploitation is a priority. For our study, we collected and extracted indigenous algal samples from marine areas of Nha Trang bay belonging to nine species of Chlorophyta, Rhodophyta, and Ochophyta to investigate their cosmeceutical activities. Results revealed tyrosinase inhibitory activity of methanolic extracts of *Halimeda* sp. and *Ulva lactuca* with 57.17 ± 1.70% and 54.32 ± 0.52% of inhibition, respectively. Additionally, methanolic extracts of *U. lactuca*, *Sargassum mcclurei*, and *S. aquifolium* were found to perform moderate scavenging capacity against DPPH free radicals. By the colorimetric method, the algae extracts were determined to exhibit no potent cytotoxic effect on both fibroblast cell line NIH-3T3 and keratinocyte cell line HaCaT at a test concentration of 20 µg/mL and thus considered promising for further safety evaluation. The investigation provides information on biological activities of our marine algae and highlights candidates with application significance.

Keywords: Marine algae, antioxidant, cosmeceutical, cytotoxicity, tyrosinase inhibition, *Ulva lactuca*.

[*]Corresponding author at: Institute of Natural Products Chemistry, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam. *E-mail addresses:* lehcuong@yahoo.com

https://doi.org/10.15625/1859-3097/18020
ISSN 1859-3097; e-ISSN 2815-5904/

INTRODUCTION

Nowadays, the development of human living quality alongside to growing demand for organic beauty products has raised continuing quests for novel cosmeceutical natural products. Natural cosmetic products tend to be safer, more biodegradable, and environmentally friendly as compared to synthetic compounds such as hydroquinone, arbutin, kojic acid, etc. Marine macroalgae have become an attractive alternative resource for supplying biomass for research needs, not only due to their high abundance and intensive distribution in almost every coastal area on the planet but also concerning their various chemical properties and biologically active ingredients are susceptible to being exploited for pharmaceutical, chemical, food, and energy industries [1]. Previous studies highlighted skin care ingredients in algae extracts, such as moisturizing polysaccharides and fatty acids of *Laminaria japonica* [2], skin whitening effect of phlorotannin isolated from *Ecklonia cava* [3], and anti-aging compounds in aqueous extract of the brown alga *Macrocystis pyrifera* [4]. Furthermore, certain algae species have been employed on an industrial scale and have been on the cosmetic market for years [5].

With a coastline of more than 3,000 km long spreading over fifteen degrees of latitude, Vietnam has significant advantages for the development of the marine cosmetic industry. However, the domestic use of macroalgae is limited, primarily as food with commonly cultivated seaweeds of genera *Caulerpa*, *Gracilaria* and *Kappaphycus* [6, 7]. Further biological surveys are, therefore, necessary for extended marine resource applications. In recent years, several algae species have been evaluated for bioactivities and formulated in skin care products, including *Caulerpa lentillifera*, *Sargassum crassifolium*, *Ulva reticulata,* and *Kappaphycus alvarezii* [8, 9], claiming the appropriateness of the research approach for tapping biotechnological potentials of seaweeds in Vietnam.

In our study, crude extracts of nine indigenous seaweeds belonging to Chlorophyta, Ochrophyta, and Rhodophyta collected from Nha Trang bay (Vietnam) were *in vitro* evaluated for cosmeceutical properties in terms of cytotoxicity, tyrosinase inhibitory and antioxidant assays. The results were expected to bring out algae candidates for potential application in cosmetic business.

MATERIALS AND METHODS

Sampling and preparation of algae extracts

Seaweeds were collected from Nha Trang bay (Khanh Hoa, Vietnam) in April 2021 and stored in seawater before being morphological identified by specialists in marine algae taxonomy from Nha Trang Institute of Technology Research and Application (MSc. Tran Mai Duc). The identification results are shown in Table 1. Algal thalli were then washed, dried, and extracted with methanol as previously described [10], yielding methanolic extracts for bioassay tests.

Cell culture

NIH-3T3 embryonic murine fibroblasts originated from the American Type Culture Collection (CRL-1658, ATCC, USA), and human keratinocyte cell line HaCaT was purchased from AddexBio (T0020001, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% streptomycin (Corning, USA), incubated at 37^{o}C, 5% CO_2 with medium change every two days.

Cytotoxicity assay

For determining the effect of algae extracts in NIH-3T3 and HaCaT cells, a sulforhodamine B (SRB) colorimetric assay was employed [11]. Cells were seeded at 4×10^4 cells per mL in 96-well microtiter plates and incubated with test extracts at 37^{o}C (72 h, 5% CO_2). The absorbance at 564 nm will be recorded (Tecan F150, Austria) for calculating the cell survival percentage in each well, as mentioned in our previous study [12].

Table 1. Algae samples' symbolization and sampling coordinates

No.	Symbol	Species name	Taxon details		Sampling site (Coordinates)
			Phylum	Class	
1	*C*1	*Halimeda* sp.	Chlorophyta	Halimedaceae	12°13.848'N, 109°14.505'E
2	*C*34	*Ulva lactuca*		Ulvaceae	12°12.473'N, 109°12.946'E
3	*O*2	*Padina australis*	Ochrophyta	Dictyotaceae	12°13.848'N, 109°14.505'E
4	*O*3	*Turbinaria ornata*		Sargassaceae	12°10.947'N, 109°17.585'E
5	*O*5	*Hormophysa cuneiformis*		Sargassaceae	12°10.732'N, 109°16.662'E
6	*O*14	*Sargassum aquifolium*		Sargassaceae	12°13.848'N, 109°14.505'E
7	*O*26	*Sargassum mcclurei*		Sargassaceae	12°10.947'N, 109°17.585'E
8	*R*8	*Hydropuntia eucheumatoides*	Rhodophyta	Gracilarioideae	12°10.732'N, 109°16.662'E
9	*R*9	*Tricleocarpa cylindrica*		Florideophyceae	12°13.848'N, 109°14.505'E

Tyrosinase inhibitory activity

Algae extracts were spectrophotometrically assayed for tyrosinase inhibitory activity following described method [13] with modifications. Extracts were dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Germany) and assayed on 96-well plates, using L-tyrosine (Sigma-Aldrich, Germany) as the substrate. Test samples were pre-incubated at 37°C for 5 mins with 100 U/mL tyrosinase (Sigma-Aldrich, Germany) in 0.05 M phosphate buffer (pH = 6.5). After 100 µL of substrate solution (L-tyrosine 2 mM in phosphate buffer) was added to the mixture and *en masse* incubated (37°C, 60 min), the absorbance was measured at 475 nm by a Tecan reader (Tecan F150, Switzerland). Kojic acid (Sigma-Aldrich, Germany) was used as a reference reagent. The control sample was prepared similarly, with the algal extract replaced by a buffer. The following equation calculated tyrosinase inhibition activity:

$$TI(\%) = \left\{1 - \left[\left(A_S - A_B\right)/\left(A_C - A_N\right)\right]\right\} * 100\%$$

where: A_S and A_B are absorbances of the test sample with and without enzyme, respectively; A_C and A_N are the control sample with and without enzyme, respectively. The IC_{50} value was defined as the concentration of specimens required for 50% inhibition of tyrosinase.

Antioxidant assay

The antioxidant potential of methanolic crude extracts was assayed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical [14] and performed on 96-well plates as presented in our previous publication [15]. Concisely, a mixture of dissolved extract solution in DMSO and DPPH radical solution 0.3 mM in absolute ethanol (Merck, Germany) was prepared and incubated at 37°C for 30 min. The color changes after reaction were recorded at 517 nm using a spectrophotometer (Spark® Cyto multi-well plate reader, Tecan, Switzerland). DMSO and ascorbic acid were used as negative and positive controls, respectively.

Statistical analysis

Experiments were carried out in triplicate, and all data are expressed as mean ±SD (Standard Deviation). The half maximal inhibitory concentration (IC_{50}) was estimated

by nonlinear regression analysis using the computer software TableCurve 2D version 5.1.

RESULTS AND DISCUSSION

Tyrosinase inhibitory activity

Tyrosinase is the key enzyme responsible for the melanin synthesis within the melanosomes of mammalian skin, which is the primary cause of skin darkening and related disorders such as melasma, freckles, and lentigines. The inhibition of tyrosinase is thus the most straightforward approach for controlling hyperpigmentation and has become a great study interest in cosmeceutics. To investigate the tyrosinase inhibitory activity of collected seaweed samples, we measured the L-tyrosine oxidation rates by detecting optical density at 475 nm after incubation with algae extracts. The results are shown in Table 2.

Table 2. Inhibitory activity of algae extracts against tyrosinase

No.	Sample	Concentration (µg.mL^{-1})	Tyrosinase inhibition activity (%)	IC_{50} (µg.mL^{-1})
1	*C*1	500	57.17 ± 1.70	383.13
2	*C*34	500	54.32 ± 0.52	377.43
3	*O*2	500	2.05 ± 0.13	ND*
4	*O*3	500	13.62 ± 1.41	ND
5	*O*5	500	7.75 ± 0.96	ND
6	*O*14	500	1.64 ± 0.08	ND
7	*O*26	500	48.11 ± 1.43	ND
8	*R*8	500	5.25 ± 1.05	ND
9	*R*9	500	11.97 ± 1.18	ND
	DMSO		0	-
	Kojic acid	50	80.73 ± 0.24	6.85

Note: ND: not determined.

According to Table 2, in the presence of test extracts, tyrosinase activity was inhibited at different levels but relatively weak compared to positive control Kojic acid (IC_{50} value of 6.85 µg/mL). Among the extracts, the most potent inhibiting percentages of 57.17% and 54.32% were observed by incubating with *C*1 (from *Halimeda* sp.) and *C*34 (from *U. lactuca*), respectively. At 500 µg.mL^{-1}, *O*26 from *S. mcclurei* inhibited nearly 50% of enzyme activity after 60 mins of reaction. Other samples presented poor tyrosinase inhibitory activity with less than 15%.

Natural tyrosinase inhibitors are continuedly applied in the beauty industry as anti-browning and skin-whitening agents. Marine macroalgae are a great source of natural tyrosinase inhibitor agents has been claimed and well studied. Common reported seaweeds were mostly brown algae (Ochrophyta), especially those belonging to Sagassaceae, such as *Sargassum silquastrum*, *Laminaria japonica*, *Ishige okamurae*, *Hizikia fusiformis*, and *Ecklonia cava* [3, 5]. *Symphyocladia latiuscula* (Rhodomelaceae) and *Gloiopeltis furcata* (Florideophyceae) are among the most described red algae (Rhodophyta) with anti-tyrosinase activity [1, 5]. Our present study revealed that methanolic extracts of green algae *C*1 and *C*34 (Chlorophyta) exhibit more outstanding inhibition rates. The discrepancies among these and previous results may be due to differences in concentrations of test extracts, experimental conditions, local environmental factors, and habitats of algal specimens.

In vitro antioxidant acitivity

In our study, the presence of antioxidative components in crude extracts of seaweeds was determined by a free radical DPPH scavenging assay. The *in vitro* method is considered relatively inexpensive and sensitive for the detection of natural antioxidants in plant and microbial samples. The antioxidant activity of

algae extracts was determined based on inhibition percentages at concentrations ranging from 5 µg.mL^{-1} to 500 µg.mL^{-1} and the *SC*50 values resulting from linear regression analysis. Figure 1 depicted the scavenging capacity percentages of extracts at 500 µg.mL^{-1} in comparison to a positive control (ascorbic acid, 50 µg.mL^{-1}).

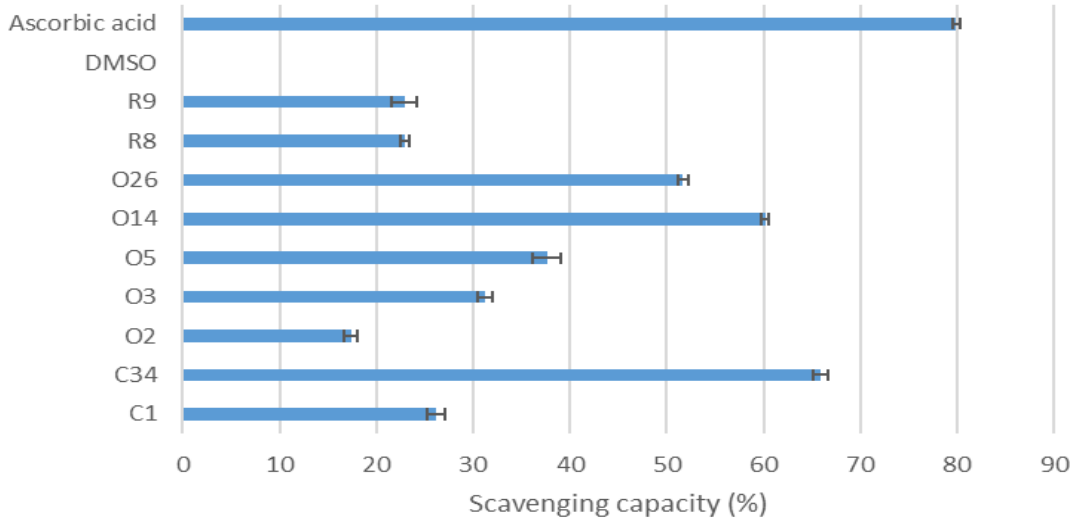

Figure 1. Free radical DPPH scavenging capacity of algae extracts

As shown in Figure 1, the methanolic extracts of algae exhibited antioxidant activity at different levels. At a test concentration of 500 µg.mL^{-1}, only three out of nine extracts were found with scavenging capacity exceeding 50%. Among these, remarkable antioxidant activity with 65.84±0.77% of scavenged free radicals (*SC*50 value of 334.28 µg/mL - data not shown) was recorded with test sample *C*34 extracted from the thallus of *U. lactuca*. Besides, moderate scavenging percentages at 60.18 ± 0.38% and 51.56 ± 0.53% by *O*14 (from *S. aquifolium*) and *O*26 (from *S. mcclurei*), respectively, were observed. These results are relatively consistent with the antioxidant activities of seaweed samples mentioned in our previous research [15]. Accordingly, extracts of algae collected from northern coastal areas of Vietnam by chloroform and methanol solvent system exhibited no potent scavenging activity against radical DPPH [15]. On the other hand, algae samples belonging to Rhodophyta tended to be less antioxidative than those belonging to Chlorophyta and Ochrophyta (Figure 1). Hypothetically, the presence of most antioxidant active compounds such as polyphenols and phlorotannins in extracts of brown and green seaweeds were at higher amounts than in green algae.

Antioxidants, especially natural sources, have been known to play an essential role in repairing and renewing skin cells and are required in effective cosmetic formulations [5]. Obtained results highlight the ability of native algae species as a natural source of antioxidant additives in cosmetic applications.

Cytotoxic effects of algae extracts

We evaluated the cytotoxicity of methanolic extracts from dried thalli of nine algae species by colorimetric test on both the murine fibroblast cell line NIH-3T3, which is known as a component of mammalian skin architecture [16] and the human keratinocyte cell line HaCaT which has been considered a model of organotypic skin [17]. The effects of test extracts at a concentration of 20 µg.mL^{-1} on NIH-3T3 and HaCaT cells' viability measured via the assay are shown in Table 3.

As presented in Table 3, at a test concentration of 20 µg.mL^{-1} and after a 72-hour incubation period, 8/9 of algae

exhibited no apparent toxic effects on fibroblasts and keratinocytes, with cell survival percentages greater than 70%. Furthermore, some of these extracts induced mild proliferation in cell line NIH-3T3 with high cell survival percentages, i.e., *C*34 (100.11 ± 0.87%), *O*26 (103.25 ± 0.35%), and *R*8 (101.81 ± 1.26%) extracted from green alga *U. lactuca*, brown alga *S. mcclurei* and red alga *H. eucheumatoides*, respectively. The result suggests the potential of our indigenous marine algae for dermatologic purposes, especially for regenerating connective tissue in the skin extracellular matrix. Bioactive dermal cosmetic ingredients may risk human skin's safety by intimate application. *In vitro* cytotoxic assessments are thus a recommended and appropriate step for biological screenings of new products.

Table 3. Cytotoxic effect of algae extracts on NIH-3T3and HaCaT cell lines

No.	Sample	Cell survival percentage (%)		IC_{50} (µg.mL^{-1})	
		NIH-3T3	HaCaT	NIH-3T3	HaCaT
	DMSO	100	100	-	-
1	*C*1	89.92 ± 1.22	90.33 ± 0.94	ND	ND
2	*C*34	100.11 ± 0.87	97.80 ± 1.24	-	ND
3	*O*2	93.15 ± 0.23	86.35 ± 1.36	ND	ND
4	*O*3	70.73 ± 0.57	83.22 ± 1.08	ND	ND
5	*O*5	99.05 ± 0.13	97.05 ± 0.13	ND	ND
6	*O*14	98.62 ± 1.18	72.91 ± 0.64	ND	ND
7	*O*26	103.25 ± 0.35	93.78 ± 0.35	-	ND
8	*R*8	101.81 ± 1.26	100	-	ND
9	*R*9	48.35 ± 1.30	67.40 ± 1.82	21.05	ND

Note: ND: not determined.

Taken screening results from tyrosine inhibitory, antioxidant, and cytotoxic assays together, extracts *C*34 (from *U. lactuca*) and *O*26 (from *S. mcclurei*) deemed it necessary to be further studied with cosmeceutical guidance. Furthermore, *U. lactuca* could be a prospective source for exploitation regarding its abundant distribution in areas of Nha Trang bay. Besides, some species, such as tyrosinase inhibiting *Halimeda* sp. and fibroblasts' proliferation-inducing *Hydropuntia eucheumatoides*, are described for the first time for cosmeceutical properties. These results would be helpful for the development of safe and effective skincare cosmetic formulations from the seaweeds of Vietnam. Obstacles await massive production and commercially application of these algal candidates as beauty products. First, the cost for extraction from raw seaweed materials makes the preparation process of active ingredients challenging. Besides, further experimental evidence, such as additional *in vitro* assays in melanoma cell lines and three-dimensional human skin models, as well as *in vivo* assays in test animal and clinical trials, are obligated to be fulfilled.

CONCLUSION

In summary, this study revealed miscellaneous tyrosine inhibitory, antioxidant, and cytotoxic properties of nine algae species belonging to Chlorophyta, Ochrophyta, and Rhodophyta collected from Nha Trang bay. Accordingly, seaweeds *Halimeda* sp., *Ulva lactuca*, *Sargassum aquifolium*, *S. mcclurei*, and *Hydropuntia eucheumatoides* appeared as probable sources of cosmeceutical active ingredients. Mainly, methanolic extract from widely distributed green alga *U. lactuca*'s thallus was found to exert moderate tyrosinase inhibiting activity, free radical scavenging capacity, and exhibited mild proliferation in fibroblast cells, therefore could be considered a promising candidate for further cosmeceutical guided evaluation and elaboration.

Acknowledgements: This study was financed by the Vietnam Academy of Science and Technology under the project code VAST 06.03/21–22.

REFERENCES

[1] Fleurence, J., and Levine, I. (Eds.), 2016. Seaweed in health and disease prevention. *Academic Press*.

[2] Choi, J. S., Moon, W. S., Choi, J. N., Do, K. H., Moon, S. H., Cho, K. K., Han, C.-J., and Choi, I. S., 2013. Effects of seaweed *Laminaria japonica* extracts on skin moisturizing activity in vivo. *J. Cosmet. Sci*, *64*(3), 193–205.

[3] Yoon, N. Y., Eom, T. K., Kim, M. M., and Kim, S. K., 2009. Inhibitory effect of phlorotannins isolated from Ecklonia cava on mushroom tyrosinase activity and melanin formation in mouse B16F10 melanoma cells. *Journal of Agricultural and Food Chemistry*, *57*(10), 4124–4129. https://doi.org/10.1021/jf900006f

[4] Thomas, N. V., Manivasagan, P., and Kim, S. K., 2014. Potential matrix metalloproteinase inhibitors from edible marine algae: A review. *Environmental Toxicology and Pharmacology*, *37*(3), 1090–1100. https://doi.org/10.1016/j.etap.2014.04.011

[5] Couteau, C., and Coiffard, L., 2016. Seaweed application in cosmetics. In *Seaweed in health and disease prevention* (pp. 423–441). *Academic Press*.

[6] Hong, D. D., Hien, H. M., and Son, P. N., 2007. Seaweeds from Vietnam used for functional food, medicine and biofertilizer. *Journal of Applied Phycology*, *19*, 817–826. https://doi.org/10.1007/s10811-007-9228-x

[7] Nguyen Van, T., 2014. Seaweed diversity in Vietnam, with an emphasis on the brown algal genus *Sargassum*. *Doctoral dissertation, Ghent University*.

[8] Hong, D. D., Vy, P. B., Thu, N. T. H., Khoi, T. X., Ha, N. C., and Tam, L. T., 2018. Evaluation of bioactivities and formulation of face mask from *Sargassum* sp. extract. *Academia Journal of Biology*, *40*(3), 106–112. https://doi.org/10.15625/2615-9023/v40n3.12686

[9] Thu, N. T. H., Anh, H. T. L., Hien, H. T. M., Ha, N. C., Tam, L. T., Khoi, T. X., Duc, T. M., and Hong, D. D., 2018. Preparation and evaluation of cream mask from Vietnamese seaweeds. *Journal of Cosmetic Science*, *69*(6), 447–462.

[10] Chi, H. K., Van, N. T. H., Hang, T. T. N., Duc, T. M., and Ha, T. T. H., 2020. Intracellular reactive oxygen species scavenging effect of fucosterol isolated from the brown alga *Sargassum crassifolium* in Vietnam. *Vietnam Journal of Marine Science and Technology*, *20*(3), 308–316. https://doi.org/10.15625/1859-3097/20/3/15248

[11] Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S., and Boyd, M. R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. *JNCI: Journal of the National Cancer Institute*, *82*(13), 1107–1112. https://doi.org/10.1093/jnci/82.13.1107

[12] Chi, H. K., Ha, T. T. H., Hang, T. T. N., and Tuan, N. D., 2018. Antimicrobial, cytotoxic and hemolytic activities of marine algae-associated fungal isolates in Vietnam. *Vietnam Journal of Marine Science and Technology*, *18*(4), 406–412. doi: 10.15625/1859-3097/18/4/13660

[13] Khatib, S., Nerya, O., Musa, R., Shmuel, M., Tamir, S., and Vaya, J., 2005. Chalcones as potent tyrosinase inhibitors: the importance of a 2,4-substituted resorcinol moiety. *Bioorganic & medicinal chemistry*, *13*(2), 433–441. https://doi.org/10.1016/j.bmc.2004.10.010

[14] Brand-Williams, W., Cuvelier, M. E., and Berset, C. L. W. T., 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

[15] Ha, T. T. H., Tuan, N. D., Chi, H. K., Hang, T. T. N., Ly, D. T. P., Imbs, A. B., and Long, P. Q., 2019. Evaluation of biological activities of some seaweed and

seagrass species in the coastal area of Vietnam. *Vietnam Journal of Marine Science and Technology*, *19*(3), 405–414. https://doi.org/10.15625/1859-3097/19/3/14060

[16] Song, Y. H., Zhu, Y. T., Ding, J., Zhou, F. Y., Xue, J. X., Jung, J. H., Li, Z. J., and Gao, W. Y., 2016. Distribution of fibroblast growth factors and their roles in skin fibroblast cell migration. *Molecular Medicine Reports*, *14*(4), 3336–3342. https://doi.org/10.3892/mmr.2016.5646

[17] Boelsma, E., Verhoeven, M. C., and Ponec, M., 1999. Reconstruction of a human skin equivalent using a spontaneously transformed keratinocyte cell line (HaCaT). *Journal of investigative dermatology*, *112*(4), 489–498. https://doi.org/10.1046/j.1523-1747.1999.00545.x