MINISTRY OF EDUCATION AND TRAINING

VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY

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DETERMINING THE ABILITY OF ASTAXANTHIN EXTRACTED FROM *HAEMATOCOCCUS PLUVIALIS* ALGAE ON PROTECTING SKIN FROM PHOTOAGING ON MOUSE MODEL

Specilization: Biotechnology

Code: 9 42 02 01

SUMMARY OF DOCTORAL DISSERTATION

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TechnologThe project is completed at: University of Sciences, Vietnam National University, Ho Chi Minh City and the Graduate University of Sciences and Technology- Vietnam Academy of Science and Technology

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INTRODUCTION

Important of subject

Astaxanthin is a carotenoid with the formula 3,3'-dihydroxy-β-carotene-4,4'-dione. Astaxanthin has the leading antioxidant activity today (100 times more than α-tocopherol and 10 times more than the compounds zeaxanthin, lutein, canthaxanthin, β-carotene). Therefore, astaxanthin is used a lot in medicine to treat diseases caused by oxidation: cardiovascular, joint, diabetes,...

Reactive oxygen species (ROS) are one of the main causes of skin aging. The amount of ROS has the ability of the skin to increase with the physiological aging process as well as being affected by external factors such as ultraviolet rays (UV rays), dust. These ROS disrupt skin cells (fibroblasts, keratinocytes) and molecules in the skin matrix such as collagen and elastin, and prevent new synthesis of these molecules. AST is a strong antioxidant capable of absorbing these free radicals, so it will inhibit the skin aging process and restore aging skin.

In the cosmetic field, astaxanthin is used in two forms: lotion and oral. Although many products are commercially available, research on astaxanthin's use in the cosmetic field is limited. The studies are fragmented, inconsistent and only conducted on a small scale. Therefore, the recommendation to use astaxanthin in the most effective way in the field of cosmetology is not yet available.

Currently, in Vietnam, astaxanthin studies have been conducted but the results are still limited, especially applied studies.

Overall Objective: *Evaluation of the anti-aging effect of astaxanthin-rich Haematococcus pluvialis extract on cell and mouse models.*

Specific objective

- Successful induction of astaxanthin synthesis from Haematococcus pluvialis
- The cell protection efficacy of the astxanthin-rich extract from the oxidizing agent was assessed under in vitro conditions.
- Evaluate the skin protection effect of astaxanthin-rich extract from UV rays.

1. BACKGROUND

1.1. *Overview of microalgae H. pluvialis*

Introduction to the morphological characteristics and life cycle of H. pluvilias and introduce the transition conditions between microalgae life cycle.

1.2. Introduction of AST

This section introduces AST: structure, synthesis pathway, properties of AST

1.3. The sources of AST

This section introduces the current AST sources such as: *H. pluvialis*, salmon, and fungi.

1.4. Anti-aging mechanism of AST

This section introduces the anti-aging properties of AST, the applications of ASX such as anti-inflammatory, DNA repair, cell protection.

1.5. Structure of the skin

This section introduces the structure and composition of the skin: 3-layer structure, including fibroblast cells, epidermal cells, keratinocytes

1.6. Skin aging

This section is about UV-induced skin aging, the effects of UV rays on skin cells and skin structure, and an introduction to cellular aging.

1.7. Cellular senescence

2. MATERIAL-METHOD

2.1. Thiết kế thí nghiệm: phương pháp thực nghiệm mô tả.

2.2. Fresh sample

- Algae *H. pluvialis*: LC strain (HP-C), Institute of Biotechnology (IBT), Vietnam Academy of Science and Technology (VAST)
- Human fibroblast cells (hF): frozen hF cells (Passage 8).
- (Mus musculus var. Albino): female, weighing from 20-25 g, about 4-6 weeks old, provided by Institute Pasteur, Ho Chi Minh City

2.3. Chemicals

- Medium for the algae cultivation: BG-11
- Cell culture medium DMEM/F12 completed 10% FBS.
- Medium contains aging agent: DMEM/F12 completed H_2O_2 150 µM and 3% FBS.

2.4. Overall experimental diagram

2.5. Research method

2.5.1. *Design of algae cultivation system*

2.5.

Direction of air flow: ⟶ Damper:
(1): dust air filter cotton bottle, (2): water bottle, (3): algae cultivation bottle

Figure 2. 2. System of Cultivating Algae

Algae Cultivating system is installed according to the diagram of Figure 2.2 with room temperature at 25^{o}C, long LED light.

2.5.2. *Method cultivating algae in the 1000 ml culture bottle*

HP-C algae were inoculated into a 1000 ml culture bottle containing 500 ml of BG-11 medium with an initial density of 10x104 cells/ml and cultured at 25°C, 3000 lux (3 klux) light conditions. light:dark 12h:12h, mild aeration conditions 2L/min, gently shake 3 times/day, culture for 15 days.

2.5.3. *Method of inducing HP-C algae to synthesize AST by strong light intensity*

Algae were inoculated into culture bottles with an initial density of 50x104 cells/ml. Algae were illuminated at 10 klux with a 24:0 h light:dark cycle. After 24 days, the algae were collected and the concentration and total AST were assessed.

2.5.4. Method for extraction AST

Collect biomass of algal cysts and dry according to each experimental group.

HCl treatment step: 50 mg of biomass was placed in 5 ml of 2N HCl solution for 1 min, centrifuged 3 times to remove HCl, then the biomass was freeze-dried at 4oC for 1 day.

Homogenization step in solvent: Weigh 50 mg of algae biomass in each group, add 10 ml of dichlormethane/methanol solution, carry out mechanical homogenization at 10000 rpm for 10 minutes to collect the solution, the residue is separated. and centrifuged to a transparent extract.

2.5.5. Method for quantitative AST

Dried algae powder was mixed with methanol and saponified with 0.107 M NaOH in absolute methanol with a volume ratio of 1 ml of NaOH: 5 ml of solution, aerated to 5 ml, incubated in the dark at 4oC, for 6 hours. Then, AST was assessed by HPLC.

2.5.6. Method for obtaining AST-rich algae extract of H. pluvialis (AST-EX)

Purpose: to collect the extract of algae H. pluvialis

Principle: extraction procedure is determined according to the optimal process evaluated in section 2.5.7

The AST-rich algal extract H. pluvialis is denoted AST-EX and is associated with a total AST concentration. Example: AST-EX 5 µg/ml is an extract of H. pluvialis with a total AST concentration of 5 µg/ml.

2.5.7. FRAP method

Prepare FRAP solution: acetate buffer 300 mmol/l, pH 3.6. TPTZ 10 mmol/l mixed in HCl 40 mmol/l. FeCl3 20 mmol/l. Add 190 µl of FRAP solution to plate -96 well, add 10 µl of AST or Trolox extract. The mixture was incubated against light for 2 h, and absorbance was measured at 593 nm. Oxidation capacity of AST will be calculated according to FRAP value: FRAP value = $A_0/A_1 \times N$, where A_0, A_1: OD value of the sample, $FeSO_4$ at 593 nm.

2.5.8. ABTS method

Prepare a solution of ABTS+• with an OD753 value of 0.7 ± 0.02.

Prepare AST solutions of 12.5 concentrations; 25; 50; 100 and 150 µg/ml. Prepare 1 mM trolox to a concentration range of 0.05; 0.1; 0.15; 0.2 and 0.3 mM. Calculation formula: % Inhibition = [(A0 - Am)/A0] × 100% and IC50 = (50 - b)/a, where, A0 is the OD753 value of ethanol and ABTS+•. TEAC (mmol/g) = (IC50Trolox/ IC50ASX).

2.5.9. Method to evaluate the toxicity of AST-EX on human Fibroblast

hF cells were inoculated onto a 96-well plate at an initial density of 8x103 cells/well. After 1 day, replace the culture medium with the

medium according to the experimental groups. After 1 or 2 days of culture, observe cell shape by microscopy and conduct MTT method to evaluate cytotoxicity. The percentage of viable cells was evaluated according to the following formula: % live cells = OD_{590SP}/OD_{590NC} x 100

2.5.10. Method to evaluate the proliferation of fibroblasts in medium completed AST-EX

hF cells were cultured on 96-well plates at an initial density of 1x103 cells/well and grown in medium with a concentration of AST-EX 05-10 µg/ml. Conduct MTT method to evaluate cell proliferation after 11 days.

2.5.11. Scratch-wound assay to access cell migration

hF cells were inoculated onto a 96-well plate at an initial density of 10 × 103 cells/well. After 1 day, use a white tip to make an incision between the cell layers and change the medium according to experimental design. AST-EX concentration: 0.5, 1, 1.5, 5, 10 µg/ml. Negative control: The culture medium contained 0.2% DMSO. Recording images of moving cells after 3 hours, 12 hours and 24 hours, image processing using ImageJ software.

2.5.12. Phương pháp MTT để đánh giá sức sống tế bào MTT assay for cell viability

hF cells were inoculated in plate 96 at a density suitable for the experiment and incubated at 37oC, 5% CO2. Remove the old medium, add 50 µl of MTT Reagent and 50 µl of culture medium to each well, and incubate at 37°C for 3 h. Then, dissolve the crystals with 50 µl of MTT Solvent, shake the plate well for 15 min under dark conditions. Measure optical density at 590 nm

2.5.13. Methods of cellular senescence induction using hydrogen peroxide (H_2O_2)

Cells were cultured in 24-well plates at a density of 3x104 cells/well and cultured for 1 day. After 1 day, remove the medium, wash with PBS, treat cell wells at concentrations of 100, 150, 175, 200 µM for 90 minutes, 120 minutes depending on the experiment. Cells were then cultured and aging marker expression assessed to determine the optimal procedure.

2.5.14. Senescence-associated β-galactosidase staining assay

Senescence-associate β-galactosidase activity was detected using a β-galactosidase staining kit (ab102534, Abcam, US).

2.5.15. Method to evaluate the ability of AST-EX to protect cells against the aging factor H_2O_2.

The experiment was divided into a total of 8 groups based on AST-EX concentration. The group treated with AST-EX concentrations of 0.5, 1, 1.5, 5, 10 µg/ml, before treatment with 150 µM H2O2 for 90 minutes (symbols are AST-EX0.5, AST, respectively. -EX1, AST-EX1,5, AST-EX5, AST-EX10) and treatment group with commercial AST concentration of 10 µg/ml (symbol TM10).

2.5.16. Hoestch and phalloidin staining assay to evaluate cell shape

Cells were fixed with paraformaldehyde and stained with Hoestch, phalloidin according to the manufacturer's instructions

2.5.17. Method to evaluate cellular mRNA expression by real-time PCR

Collecting total RNA using the Total RNA PurificATION-EXion Kit

Perform qPCR reaction according to SyGreen 1-Step Lo-ROX kit

The expression was evaluated using the $2^{-\Delta\Delta Ct}$ formula.

Table 2. 4. Primer sequence

hMMP1-F	CAGAGATGAAGTCCGGTTTTTC
hMMP1-R	GGGGTATCCGTGTAGCACAT
hMMP3-F	CAAAACATATTTCTTTGTAGAGGACAA
hMMP3-R	TTCAGCTATTTGCTTGGGAA
$hp16^{INK4a}$ -F	TGAGCACTCACGCCCTAAGC

$hp16^{INK4a}$-R	TAGCAGTGTGACTCAAGAGAAGCC
hp21-F	GAGCACTGCCCAACAACAC
hp21-R	ATGGCGGGAGGTAGACTGA
hCOL1A1-F	AGCAGGCAAACCTGGTGAAC
hCOL1A1-R	AACCTCTCTCGCCTCTTGCT
hELN-F	TGTCCATCCTCCACCCCTCT
hELN-R	CCAGGAACTCCACCAGGAAT
hGAPDH-F	GAAGGTGAAGGTCGGAGT
hGAPDH-R	GAAGATGGTGATGGGATTTC

2.5.18. WESTERN BLOT method evaluates the expression of CDK4, CDK6 and cyclin D1

Collect total protein by cell lysis in Optiblot LDS Sample Buffer. Then, proteins were electrophoresed with Precast Gel SDS-PAGE 4-12% for 2 h at 50V, PVDF membrane hybridization and incubation of anti-CDK4, anti-CDK6, anti-cyclin D1, anti-actin antibodies. Then secondary antibody staining and identification by X-ray film.

2.5.19. Methods for design a model of skin aging on mice (In vivo)

Mices were provided by the Pasteur Institute, Ho Chi Minh City, shaving and UV irradiation according to the process of increasing intensity 1-6 MED over 8 weeks. Morphological changes in mouse skin were recorded weekly and recorded by imaging, HE histological slices on the last day of the experiment.

2.5.20. Method to evaluate the ability to protect mice skin from UVB rays

Powdered AST is mixed with organic sacha inchi oil for cosmetic use. The obtained AST was applied directly to the mouse skin according to the experimental set-up.

Experimental set-up: mices were divided into 6 groups as shown in Table 2.5. Each experiment was conducted with 4 ones

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3. RESULTS AND DISCUSSION

3.1. H. pluvialis cultivating culture and AST . induction

Growth stage: HP-C algae are green, 20 ± 3 µm. Mature follicles turned completely red, 32.61 ± 4.57 µm and the maximum AST concentration was 3.09% of dry biomass.

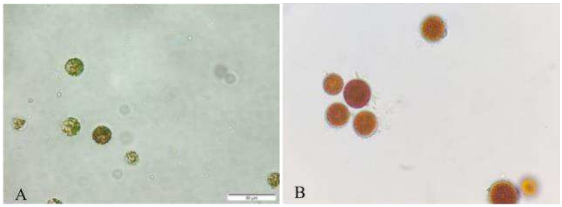

Figure 3. 1. Life cycle of HP-C algae used in the study (x40).

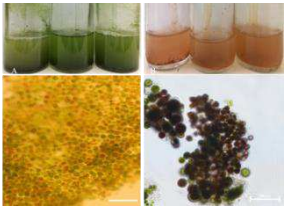

Figure 3. 2. HP-C culture results
A: proliferation culture in BG-11 medium after 26 days. B: Synthetic induction of AST in light of 10 klux. C: HP-C algae under the conditions of proliferation culture, D: algae HP-C under conditions of AST synthesis induction

3.2. Extraction of AST by solvent

The comparison between TN3 and TN1 shows that the freeze-drying method is effective in supporting the extraction of AST from algae

Table 3. 1. Summary results of AST extraction according to the procedure EX1, EX2, EX3.

(^{a}, ^{b}: statistical difference in the same row ($p<0.05$)

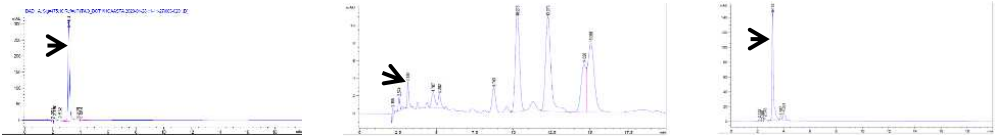

Figure 3.3 Results HPLC of *Haematococcus pluvialis* extraction

The extraction procedure by combining lyophilization and assimilation in solvent was used for all of the following experiments.

3.3. Antioxidant FRAP

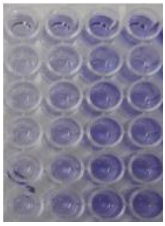

Figure 3. 5. FRAP result

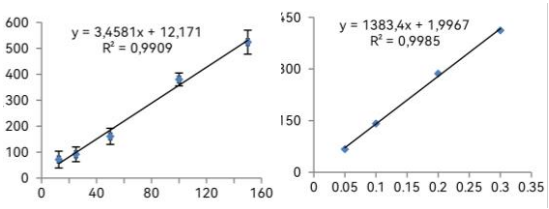

Figure 3. 6. FRAP value graph

3.4. Antioxidant ABTS

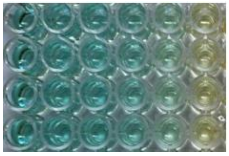

Figure 3. 7: ABTS result

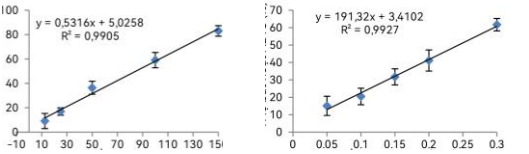

Figure 3. 8. value graph $ABTS^{+\bullet}$

3.5. I*n vitro toxicity*

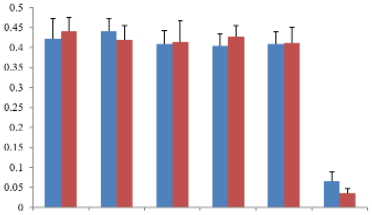

Figure 3. 9. Graph showing the OD_{495} value obtained from the AST-EX toxicity test

Bảng 3. 2. Tỉ lệ RBG của AST-EX ở các nồng độ khác nhau

100%	100%
104,27 ± 7,64%[a]	95,45 ± 7,87%[a]
97,16 ± 7,49%[a]	94,09 ± 11,91%[a]
95,73 ± 7,12%[a]	97,27 ± 6,00%[a]
97,16 ± 6,87%[a]	93,64 ± 8,79%[a]
15,64 ± 5,73%[b]	8,53 ± 2,42%[b]

According to ISO 10993-5, RBG ratio greater than 70% is non-cytotoxic solution. Therefore, AST-EX concentrations of 0.5-10 µg/ml are not cytotoxic.

3.6. Proliferation

These results confirm that AST-EX 05-1.5 µg/ml has no adverse effect on hF cell proliferation.

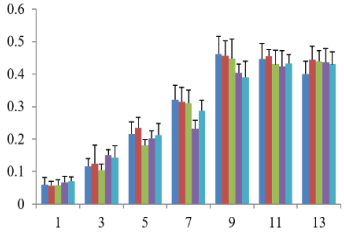

Figure 3. 10. Graph showing hF cell growth in medium completed AST-EX after 13 hour

$49,3 \pm 3,1^{a}$
$47,8 \pm 2,5^{a}$
$49,2 \pm 3,2^{a}$
$50,2 \pm 3,3^{a}$
$55,2 \pm 2,1^{ab}$
$58,8 \pm 4,7^{b}$

Table 3. 3. Proliferation time of hF cells in experimental groups

3.7. Migration

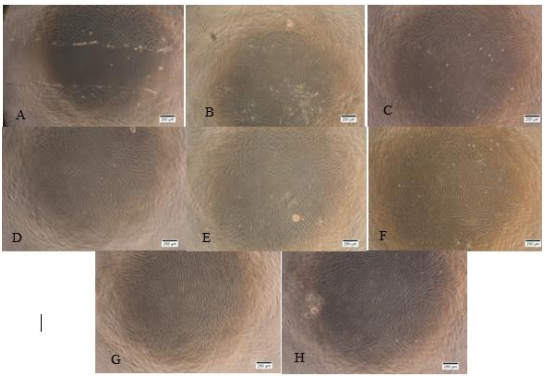

Figure 3. 11. Results of hF migration test under AST-EX 0.5-10 g/ml

Statistical processing results showed that there was no difference between groups at the same time of 3 hours, 12 hours or 24 hours. The above results showed that AST-EX 0.5-10 µg/ml did not affect the cell migration process.

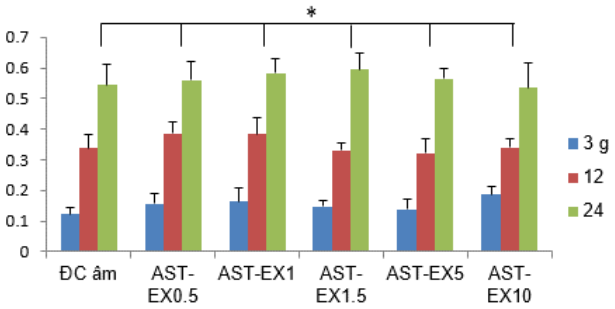

Figure 3. 12. Diagram showing the area covered by cells in the migration assay.

Table 3. 4. Results of evaluating the effectiveness of supporting hF migration after 24 hours (% of area compared to wound area)

16,3 ± 3,1[a]	44,9 ± 6,0[a]	72,3 ± 8,9[a]
20,8 ± 4,5[a]	51.5 ± 5,1[a]	74,8 ± 7,9[a]
21,9 ± 5,9[a]	51,1 ± 7,3[a]	77,9 ± 6,4[a]
19,7 ± 3,5[a]	44,1 ± 3,5[a]	79,2 ± 7,6[a]
18,4 ± 6,5[a]	42,8 ± 6,5[a]	75,2 ± 4,3[a]
24,8 ± 4,1[a]	45,2 ± 4,1[a]	71,2 ± 11,2[a]

3.8. Investigation of the process of inducing cell senescence by H_2O_2

3.8.1. Cell growth result

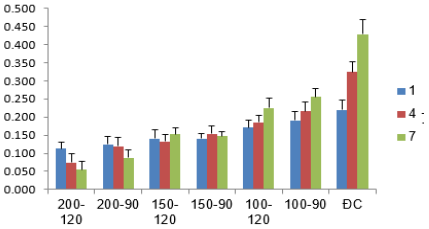

Figure 3. 13. Graph showing the increase in OD values in groups after 7 days

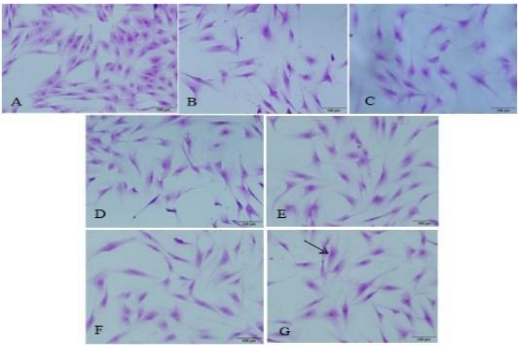

Figure 3. 14. Change of hF cell shape when treated with H2O2 on 4th day (x50). A, B, C, D, E, F, G: cells of the control groups, 100-90, 100-120, 150-90, 150-120, 200-90, 200-120. Scale bar: 100 m, arrow: enlarged cells

3.8.2. Survey results on SA-β-Gal expression

Table 3. 5. The percentage of cells positive for SA-gal when treated with H2O2 (a, b, c: statistical difference, $p<0.05$

4,60 ± 1,52[a]	47,14 ± 8,48[b]	51,44 ± 11,79[b]	65,25 ± 8,97[c]	70,73 ± 8,31[c]	72,03 ± 6,73[c]	74,99 ± 7,29[c]

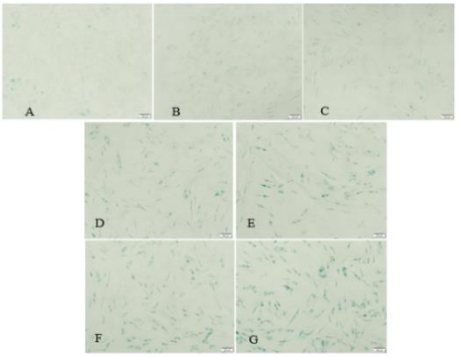

Figure 3. 15. Results of SA-gal staining of hF cells treated with H_2O_2 at different concentrations. (x100). A, B, C, D, E, F, G: cells groups: Control, 100-90, 100-120, 150-90, 150-120, 200-90, 200-120. Scale bar: 100 µm

Therefore, the concentration of 150 µM, the treatment time of 90 minutes and 120 minutes are all suitable for the set criteria.

3.9. Investigation of AST-EX's ability to protect fibroblast cells from the effects of H_2O_2

3.9.1. Results of cell area assessment

This result shows that H_2O_2 changes cell shape: cells flatten, expand cytoplasm and nucleus, while AST-EX 1-10 µg/ml limits this effect (Figure 3.26 and Table 3.26). 3.10).

Table 3. 6. Cell area and nucleus area in AST-EX and H_2O_2 treatment groups (a, b, c d: statistical difference in the same row, $p<0.05$)

	ĐC	H_2O_2	AST-EX0.5	AST-EX1	AST-EX1.5	AST-EX5	AST-EX10	TM10
Diện tích tế bào	2430,9 ± 212,6^{a}	10527,25 ± 3798,1^{b}	3919,2 ± 1172,2^{c}	3102,7 ± 1172,2^{d}	3065,8 ± 393,8^{d}	3193,2 ± 393,8^{d}	3153,1 ± 393,8^{d}	3868,9 ± 345,8^{cd}
Diện tích nhân	224,8 ± 21.4^{a}	383,3 ± 93,7^{b}	293,9 ± 27,1^{c}	255,4 ± 42,0^{a}	216,3 ± 43,5^{a}	246,2 ± 41,6^{a}	251,1 ± 38,4^{a}	285,2 ± 24,5^{c}
Tỉ lệ tế bào/nhân	11,8 ± 2.1	17,1 ± 1,6	13,2 ± 4,0	12,3 ± 2,6	14,9 ± 4,3	13,2 ± 2,0	12,8 ± 1,9	13,6 ± 4,4

3.9.2. The results of the assessment of cell proliferation

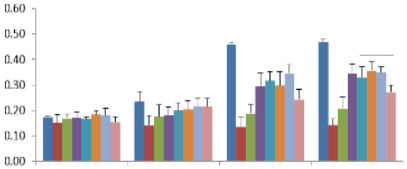

Figure 3. 17. Graph showing cell proliferation treated with AST-EX and H_2O_2 through days

This showed that H_2O_2 inhibited cell proliferation, cells in the TM group still maintained the ability to proliferate although lower than in the AST-EX group.

3.9.3. Results of evaluating SA-gal expression

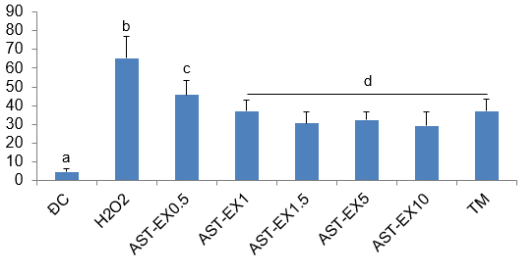

Figure 3.18. The histogram shows the percentage of cells that are positive for SA-gal

This result partly demonstrates the ability of AST-EX to protect cells from oxidative stress agent H_2O_2.

3.9.4. Results of evaluating the expression of aging markers p53, p2, p16 by realtime-PCR

The results showed that AST-EX concentration of 1, 1.5 g/ml reduced the expression of aging markers p53, p21, p16 (Figure 3.23).

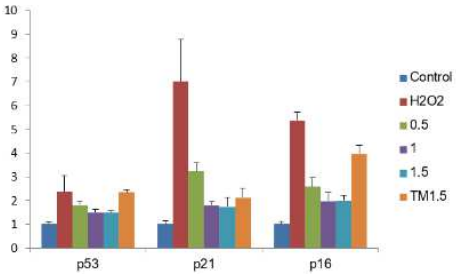

Figure 3. 20. Graph showing the expression of aging markers p53, p16, p21 in groups of cells treated with AST-EX and H_2O_2

Table 3. 9. Relative mRNA expression of p53, p21, p16 genes in the cytoprotective experiment of AST-EX (a, b, c, d: is the statistical difference in the same line) , $p<0.05$)

p53	$1,0 \pm 0,1^{a}$	$2,4 \pm 0,6^{b}$	$1,8 \pm 0,2^{b}$	$1,6 \pm 0,2^{b}$	$1,5 \pm 0,1^{b}$	$2,4 \pm 0,1^{b}$
p21	$1,0 \pm 0,2^{a}$	$7,0 \pm 1,8^{b}$	$3,2 \pm 0,3^{c}$	$1,8 \pm 0,2^{c}$	$1,8 \pm 0,3^{c}$	$2,1 \pm 0,4^{c}$
p16	$1,0 \pm 0,1^{a}$	$5,4 \pm 0,4^{b}$	$2,6 \pm 0,4^{c}$	$1,9 \pm 0,3^{c}$	$2,1 \pm 0,1^{c}$	$4,0 \pm 0,4^{d}$

3.9.5. *Results of evaluating the expression of cell function at the transcriptional level*

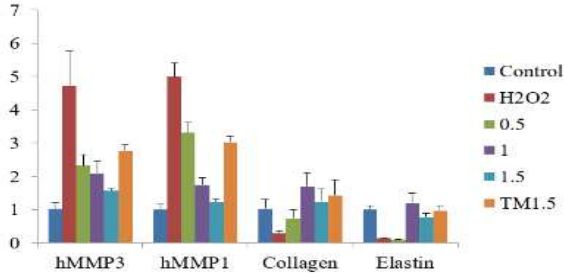

Figure 3. 21. Graph showing the expression of hMMP3, hMMP1, collagen, elastin cells treated with AST-EX

Meanwhile, cells in the AST-EX group had an increase of 2 MMP3, MMP1 mRNA compared to the control group but decreased compared with the H_2O_2 group (Figure 3.24). For collagen and elastin, the H_2O_2 cell expression decreased compared with the control group (reduced to 29% and 13% compared to the control group respectively).

3.9.6. *Results of evaluating the expression of cell function at the protein level*

Cells in the AST-EX1 group showed an increase in cylin D1 expression (1.5 times higher than the control) and a decrease compared with the cells in the H2O2 group.

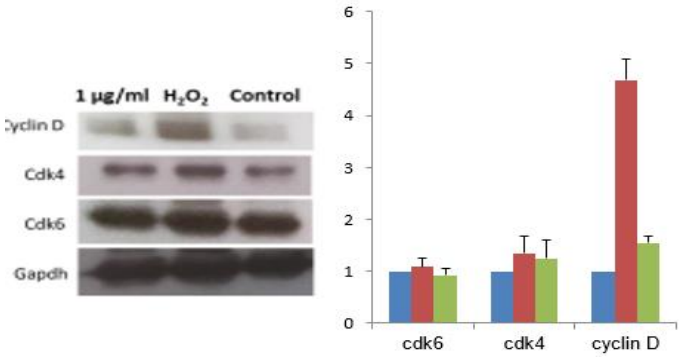

Figure 3. 22. Histogram showing the expression of CDK4,CDK6, cyclin D1 of hF cells

Table 3. 10. Results of relative expression of CDK4, CDK6 and cyclin D proteins (a,b: statistical difference in the same column, p<0.005)

CDK6	CDK4	cyclin D
1± 0.2[a]	1 ± 0.1[a]	1 ± 0.1[a]
1.1 ± 02[a]	1.3 ± 0.2[a]	4.7 ± 0.4[b]
0.9 ± 0.2[a]	1.2 ± 0.2[a]	1.5 ± 0.3[a]

The results show that AST-EX reduces the harmful effects of H_2O_2 on fibroblast cells, the concentration of 1 µg/ml is the minimum concentration that shows the cytoprotective effect.

3.10. Results of evaluating the ability to protect mouse skin from aging due to UV rays

3.11.1 Model of skin aging caused by UV rays

At week 6, wrinkles appeared across the back of the mouse, these marks were more obvious when the mouse moved. Weeks 7-8, the skin of the mouse is stretched, the skin is less elastic, so the crack becomes indistinct.

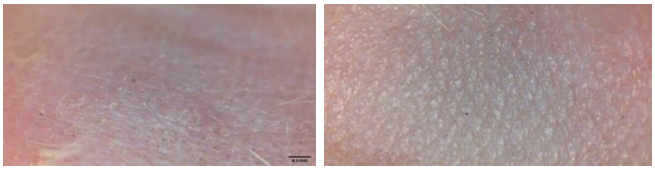

Figure 3.24. Results of mouse skin analysis after uv irradiation
A: control group, B: 8 weeks after UVB irradiation

Trichrome staining results showed that the epidermal thickness of the AST-EX group was reduced compared with the UV irradiation or oil applied group.

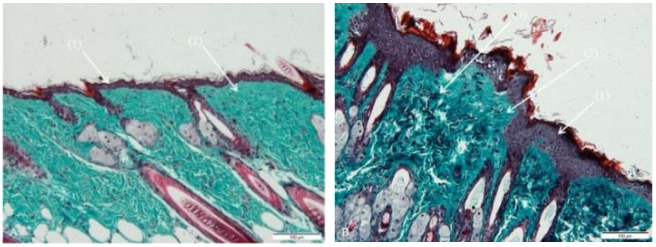

Figure 3.25. Results of trichrome staining of mouse skin samples
A: Control mouse skin sample, B: 8-week UV irradiated mouse skin sample. (1): epidermis, (2): dermis, (3): abnormal collagen structure

Table 3. 11. Summary of the results of the skin aging on mouse model caused by UVB rays. (a,b: statistical difference, p<0.05)

0.5 ± 0.5^a	4.67 ± 0.47^b
0.25 ± 0.43^a	2.5 ± 0.5^b
21.3 ± 0.8^a	65.8 ± 2.7^b
Không phát hiện	Có
Không phát hiện	Có

3.11.2 Result of AST-EX's ability to protect skin from UV damage

Table 3. 12. Summary of results for skin wrinkling and epidermal thickness. (a,b,c,d,e,f: statistical difference in the same line, $p < 0.05$)

ĐC	UVB	Sacha	AST-EX5	AST-EX10	AST-EX20	AST-EX200
0.5 ± 0.5	4.5 ± 0.5	3.5 ± 0.5	3.25 ± 0.43	3.0 ± 0.7	2.5 ± 0.5	2.25 ± 0.43
0.25 ± 0.43	2.5 ± 0.5	2.25 ± 0.43	1.75 ± 0.42	1.5 ± 0.5	1.0 ± 0.7	0.75 ± 0.43
21.4 ± 6.7^a	58.9 ± 20.9^b	54.7 ± 12.9^b	47.3 ± 10.0^c	41.7 ± 9.8^d	33.5 ± 9.6^e	28.9 ± 7.3^e
*	*****	****	**	**	*	*
*	*****	****	***	***	**	**

Dermatoscopy results show that AST-EX 10, 20, 20 has good effect in protecting the surface of the mouse skin.

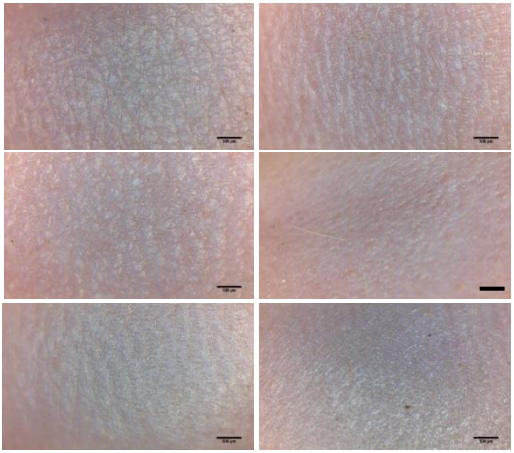

Figure 3. 27. Results of dermatoscopy after 8 weeks of UVB irradiation. A: UVB irradiation group, B: Sacha inchi oil-treated mice, C, D, E, F: AST-EX 5, 10, 20 mice, 200 g/ml

Trichrome staining results showed that, after 8 weeks of UV irradiation, there was an increase in the thickness of the epidermis, the infiltration of inflammatory cells, the formation of abnormal collagen structures (Figures 3.28, 3.29).

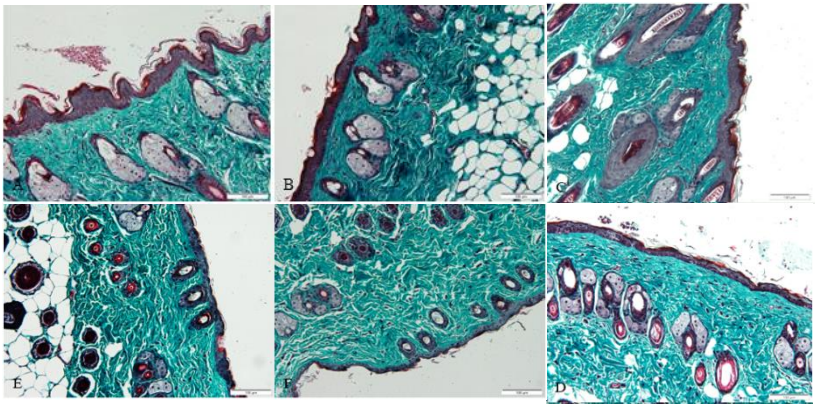

Figure 3. 28. Trichrome staining of mouse skin samples after 8 weeks of UVB irradiation. A: UVB irradiation group, B: Sacha inchi oil-treated mice, C, D, E, F: AST-EX 5, 10, 20 mice, 200 g/ml

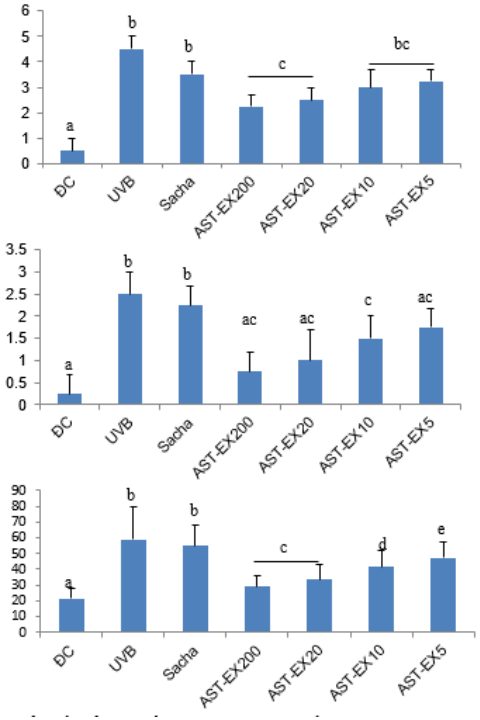

Figure 3. 29. Graph showing the results of AST-EX's anti-aging mouse skin test.
A: Histogram shows skin wrinkling, B: Histogram shows skin flaccidity, C: histogram shows epidermal thickness

4. CONCLUSION

From the results obtained from the study, we would like to conclude the following:

- Successfully induced AST from HP-C algae with the highest concentration of 3.1% dry weight.
- The AST-rich extract has the ability to protect cells from H_2O_2 agents and the concentration of 1 g/ml is the minimum concentration that has a function.
- AST-rich extract, when used in topical formulation, protected the mouse's skin from UV rays, the concentration of 20 µg/ml giving the best results.

NEW CONTRIBUTIONS OF THE THESIS

Research novelties:

- Investigate some properties of local algae *H. pluvialis* isolated in Vietnam
- Research shows that astanxanthin-rich extract from microalgae has the ability to protect cells from strong stress oxidative agent H_2O_2 in vitro and this ability is higher than pure AST. This is the basis for using AST-rich extracts for humans
- The study showed that the optimal concentration when using AST-rich extract was 20 µg/ml.

LIST OF PUBLICATIONS

Quan Minh To, Nhan Dinh Tran, Truc Thi Thanh Vo, Thao Thu Huynh, Dieu Quang Tran, Trinh Nguyen Ai Ta1, Bien Dinh Lai, Dung Hoang Nguyen, Long Thanh Le, Determining the ability of AST from H. pluvialis on the protection of skin in the mouse model, Journal of Applied Biology & Biotechnology Vol. 9(04), pp. 85-92

Quan Minh To, Dieu Quang Tran, Tuyet Thi Vy Le, Ha Le Bao Tran, Long Thanh Le, The ability of AST-rich *H. pluvialis* algal extract on preventing harmful effects of H_2O_2 on human fibroblast cells *in vitro,* European Journal of Molecular & Clinical Medicine, Volume 08, Issue 03, 2021, 1809-1919.