PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

Report Form for PhD student annual evaluation (XXXVII and XXXVIII cycles)

Name of PhD student: Alessia Luccarini

Title of PhD research: Safeguarding our skin and oceans: in quest of natural photoprotective agents from plants and marine organisms.

Name of PhD supervisor: Prof.ssa Damiani Elisabetta
Research lab name: Laboratory of Food Biochemistry, Nutrition and Oxidative Stress, DiSVA

Cycle
[ ] XXXVII
[X] XXXVIII

PhD Curriculum::
[ ] Marine biology and ecology
[X] Biomolecular Sciences
[ ] Civil and environmental protection

DISVA instrumentation labs/infrastructure eventually involved in the project:
[ ] Actea Mobile Laboratory
[X] Advanced Instrumentation lab
[ ] Aquarium
[ ] MassSpec lab
[X] MaSBiC
[ ] Simulation/informatics lab
[ ] Other. Please, indicate: .....

ABSTRACT (1000 characters, including spaces):

Despite the knowledge that sunscreens are an undeniably important tool in the fight against skin cancer, their formulations may need to be improved to contain safer ingredients, particularly regarding the concerns raised on the potential eco-toxicity of some sunscreens containing benzophenone-3 (oxybenzone) and octyl methoxycinnamate (octinoxate), that have recently come under scrutiny due to their widespread use leading to bio-accumulation and to reproductive and developmental disorders in various organisms, such that they are now banned from sale and distribution in several USA locations and nations [1, 2].
Considering this increasing danger due to the use of certain UV filters, my PhD studies aim to explore the possible photoprotective properties of different natural compounds with the aim of being able to use these compounds to gradually replace, even in part, the synthetic filters present in solar products, as innovative, natural UV filters.

Part 1. Scientific case of the PhD Research (2 to 3 pages, including figures)

- BACKGROUND

The skin is the largest organ in the human body hence skin health is inextricably linked to health and quality of life. The most common external cause of skin damage, hastening skin ageing and raising the risk of skin cancer is excessive exposure to ultraviolet radiation (UVR) from the sun, specifically UVA (315-400 nm) and UVB (280-315 nm). For decreasing the deleterious effects of UVR on the skin, one of the most widespread and extremely useful measures is the use of sunscreens that are topical products containing several different synthetic organic and inorganic UVR filters.

Nowadays, consumers are increasingly aware of the origin (synthetic or natural) and eco-sustainability of their personal care products, and recent concerns have been raised on the potential eco-toxicity of some sunscreens to the marine environment as well as to human health [3].

For example, several organic UV filters are known to induce endocrine disruption, hepatotoxicity, mutagenicity, and systemic toxicity in various organisms [4].

Therefore, turning to nature to look for natural products to replace synthetic chemicals is a growing trend in the pharmaceutical/cosmeceutical sector. In this context, photoprotective compounds derived from marine environments and extracted either from their natural sources or produced by engineering yeast or other microorganisms, such as bacteria and algae [5], represent an attractive strategy.

Furthermore, phenolic compounds are biosynthesised in plants to protect against UVR, amongst others, and the *Cyclopia spp* (Honeybush) endemic to South Africa, contain an interesting complement of polyphenols which may provide an alternative solution to synthetic benzophenones currently used in sunscreens. Phenolics also have antioxidant properties enabling them to neutralise ROS generated in the skin because of UVR exposure. Therefore, characterising the photoprotective qualities of novel benzophenones or other phenolic compounds to produce natural-based products that are safer for human use and more ecologically friendly would be an interesting approach to address concerns regarding eco-toxicity and safety [6].

A wide range of natural products are also produced in different marine species and that are mainly thought to act as a defensive strategy against UVR-mediated damage. In this context, a group of sulfur-containing compounds that are derivatives of the amino acid histidine, known as ovothiols, merit investigation, since they are found in high concentration in sea urchin eggs and exhibit unique redox properties suggestive of numerous cellular functions [7]. The marine environment thus represents a yet untapped source of naturally occurring UVR screening agents that could be used as eco-friendly and safer alternatives to synthetic UV filters.

- SCIENTIFIC AIMS

The aim of the project is to characterize and evaluate the unexplored potential of phenolics from honeybush, in particular benzophenones, but also from other plants, and of marine-inspired ovothiol compounds, endowed with both UV screening and antioxidant actions, as natural-derived, photoprotective agents against the long-term detrimental effects of excessive solar UVR on the skin.

- WORKPLAN AND RESEARCH ACTIVITIES

WP 1. Objective

The aim of this project was to investigate the potential of Honeybush (*Cyclopia spp*.) extracts, enriched in benzophenones, as photoprotective agents, which could partially substitute synthetic UV filters, such as benzophenone-3 (BP-3), that has come under scrutiny for its potential negative health and environmental effects.

Methods

- UVA irradiation
- UV spectroscopy
- HuDe cell Cultures
- Oxidative stress assays for determining oxidation levels in isolated proteins and lipids
- Flow cytometry for determining cell viability, intracellular and mitochondrial ROS production using appropriate fluorescent probes
- Fluorescence imaging

Obtained Results

- Photostability Investigation: To characterize the UV-absorbing properties and photostability of these compounds (BEF, ARC2028 and IRIFLOR), a UVA sun lamp was used as irradiation source. As seen in **Figure 1**, the spectra do not show significant changes before and after UVA exposure.

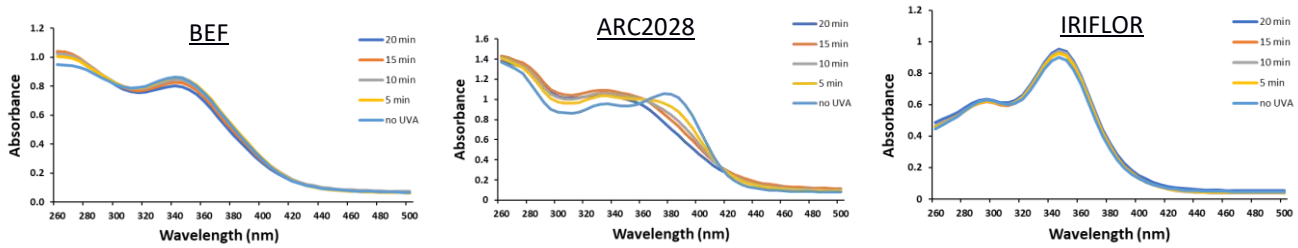

Figure 1: Absorption spectra of extracts from the Honeybush plant after different exposure times to UVA

- Shielding effect on BSA and Liposomes: To evaluate the UV-screening ability of Honeybush extract in a cell-free model, different assays were performed. Content of carbonyl group (**Figure 2A**) and levels of TBARS **(Figure 2B)** were evaluated in the presence or absence of these different compounds after UVA exposure.

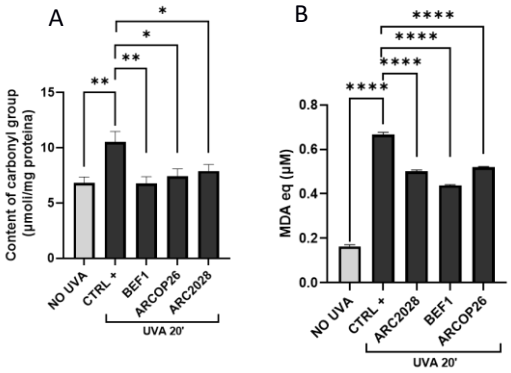

Figure 2: Oxidation levels in the presence or absence of honeybush extract (ARC2028), benzophenone-enriched fraction (BEF1) and of iriflophenone diglucoside (ARCOP26) used as shielding agents, in BSA **(A)** and PC liposomes **(B)** after 20 min UVA irradiation (~540 kJ/m^2). (One-way ANOVA: ****p<0.0001; **p<0.001; *p<0.05 vs CTRL+).

- Evaluation of the photoprotective effects on cell cultures (Human dermal fibroblasts): To evaluate the shielding effect of compounds, appropriate fluorescent probes were used, to establish cell viability (**Figure 3A**) and intracellular (**Figure 3B**) and mitochondrial (**Figure 3C**) ROS production.

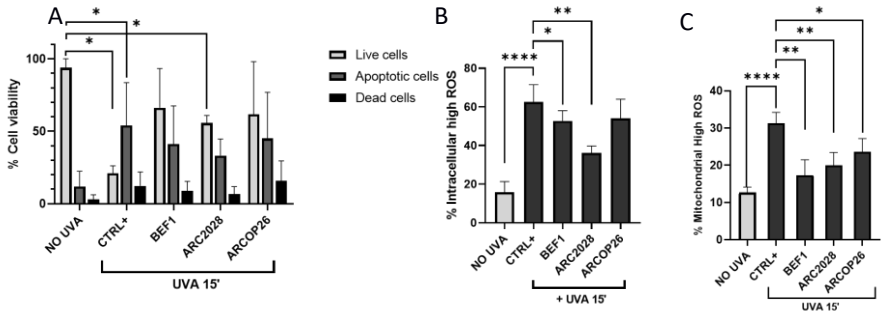

Figure 3: Cell Viability **(A)** and production of intracellular **(B)** and mitochondrial high ROS **(C)** (expressed as a percentage) in Human Dermal Fibroblasts in the presence or absence of Honeybush extract (ARC2028), benzophenone-enriched fraction (BEF1) and of iriflophenone diglucoside (ARCOP26) used as shielding agents after 15 min UVA irradiation (One-way ANOVA: ****p<0.0001; **p<0.01; *p<0.05 vs CTRL+).

The project is still ongoing.

WP 2. Objective

The awareness that the planet's resources are not inexhaustible is leading consumers to change their lifestyles, adopting behaviours and using more sustainable and safe products both for the surrounding environment and for human health. The aim of this study was to examine the possible photoprotective properties of 5-thiohistidine and iso-Ovothiol A, with the aim of being able to use these compounds to gradually replace, even in part, the synthetic filters present in solar products as innovative marine-inspired UV filters.

Methods.

- UV spectroscopy
- UVA irradiation
- Ellman's Assay
- Sodium Borohydride Assay
- Oxidative stress assays for determining oxidation levels in isolated proteins and lipids

Obtained Results

- Absorption spectra: To investigate the photostability of 5-Thiohistidine and iso-Ovothiol A, the optical absorption spectra of 5-Thiohistidine and iso-Ovothiol A (**Figure 1A**) were analysed before and after 20 min UVA exposure. As shown in **Figure 1B-C**, UVA exposure induces a change in the absorption spectrum; in fact, starting from 320 nm an increase in the absorbance value is observed in both compounds compared to those not exposed to UVA.

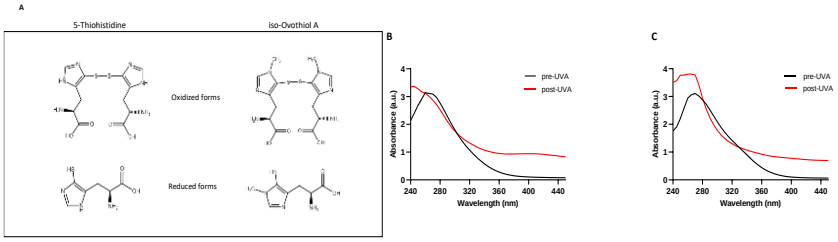

Figure 1. Chemical structures of the oxidized and reduced forms (A) of the two marine-inspired thiol compounds; optical absorption spectra of 5-Thiohistidine (B) and iso-Ovothiol A (C) not exposed and exposed to 20 min UVA (540 kJ/m^2).

- Ellman's Assay: To establish the oxidation state of these compounds before and after UVA exposure, Ellman's Assays was performed. **Table 1** shows that compounds not exposed to UVA have an absorbance value around zero, suggesting their oxidized form. On the other hand, subsequent to UVA exposure there is a significant increase in absorbance suggesting their reduced form. GSSG and GSH were also tested for comparison.

	Absorbance at 412 nm	
	pre- UVA	post- UVA 20 min
GSSG	-0.055 ± 0.016	-0.041 ± 0.007
GSH	0.522 ± 0.004	0.481 ± 0.020
5-Thio	0.0035 ± 0.001	0.939 ± 0.016 **
i-OvoA	- 0.065 ± 0.021	0.601 ± 0.025 **

Table 1. Measurement at 412 nm of 5-Thiohistidine and iso-Ovothiol A before and after 20 min UVA exposure. Both oxidized and reduced Glutathione were used as a comparison. The results are expressed as mean value ± S.D. (*n* = 3). ** p < 0.001 vs pre-UVA.

- Sodium Borohydride assay: To confirm the role of UVA exposure in the reduction of these compounds, sodium borohydride reagent was used. Both 5-Thio and i-OvoA undergo an increase in absorbance value (not shown), which confirms the results obtained in Figure 1B-C.
- Shielding effect on BSA and Liposomes: To investigate the shielding effect of the two marine-inspired compounds, the fluorescence intensity of Tryptophan in BSA (**Figure 3A**) was measured after 20 min UVA exposure, in the presence or absence of 5-Thio and i-OvoA not previously irradiated, to demonstrate that photoprotection is achieved only by the reduced forms. The content in carbonyl groups and level of TBARS were also measured using the pre-irradiated forms; as shown (**Figure 3B**), the degree of protein carbonyl formation in BSA and the level of TBARS in PC liposomes **(Figure 4B)** after 20 min UVA exposure in the presence of these compounds, was significantly decreased compared to the positive control (0).

  The project is still ongoing.

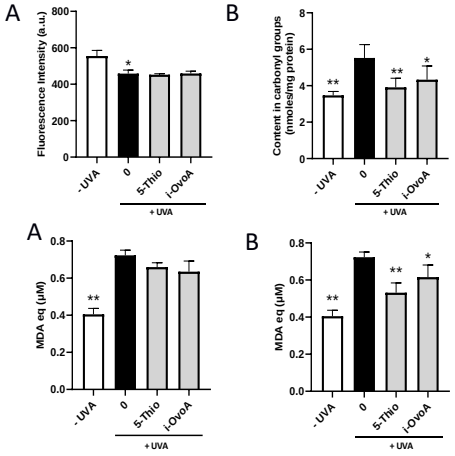

Figure 3. Fluorescence intensity of tryptophan **(A)** in the presence or absence of not pre-irradiated 5-thio and i-OvoA (0.9 mM) used as shielding agents, exposed to 20 min UVA irradiation (540 kJ/m^2). Content in carbonyl groups **(B)** after 20 min UVA exposure using pre-irradiated compounds (0.9 mM) as shielding agents. Error bars represent ± S.D. *p < 0.05; p < 0.001 vs -UVA (not exposed).

Figure 4. Oxidation levels in PC liposomes in the presence or absence of 5-Thio and i-OvoA (0.9 mM) used as shielding agents in the oxidized **(A)** and in the reduced forms **(B)** exposed to 20 min UVA irradiation (540 kJ/m^2). Error bars represent ± S.D. *p < 0.05, **p < 0.001 vs -UVA (not exposed).

- REFERENCES

[1] Agawin, N. S., Sunyer-Caldú, A., Díaz-Cruz, M. S., Frank-Comas, A., García-Márquez, M. G., & Tovar-Sánchez, A. (2022). Mediterranean seagrass Posidonia oceanica accumulates sunscreen UV filters. *Marine pollution bulletin*, *176*, 113417.

[2] Oral, D., Yirun, A., & Erkekoglu, P. (2020). Safety concerns of organic ultraviolet filters: special focus on endocrine-disrupting properties. *Journal of Environmental Pathology, Toxicology and Oncology*, *39*(3).

[3] Corinaldesi, C., Marcellini, F., Nepote, E., Damiani, E., & Danovaro, R. (2018). Impact of inorganic UV filters contained in sunscreen products on tropical stony corals (Acropora spp.). *Science of The Total Environment*, *637*, 1279-1285.

[4] Oral, D., Yirun, A., & Erkekoglu, P. (2020). Safety concerns of organic ultraviolet filters: special focus on endocrine-disrupting properties. *Journal of Environmental Pathology, Toxicology and Oncology*, *39*(3).

[5] Corinaldesi, C., Damiani, E., Marcellini, F., Falugi, C., Tiano, L., Brugè, F., & Danovaro, R. (2017). Sunscreen products impair the early developmental stages of the sea urchin Paracentrotus lividus. *Scientific reports*, *7*(1), 7815.

[6] Joubert, E., De Beer, D., Malherbe, C. J., Muller, M., Louw, A., & Gelderblom, W. C. A. (2019). Formal honeybush tea industry reaches 20-year milestone–progress of product research targeting phenolic composition, quality and bioactivity. *South African Journal of Botany*, *127*, 58-79.

[7] Castellano, I., & Seebeck, F. P. (2018). On ovothiol biosynthesis and biological roles: from life in the ocean to therapeutic potential. *Natural product reports*, *35*(12), 1241-1250.

Part 2. PhD student information on the overall year activity (courses/seminars/schools, mobility periods, participation to conferences)

List of attended courses/seminars/schools

1. Design of Research (European Projects) – Prof. Paone Nicola

2. Technology Transfer and Innovation – Prof. Iacobucci Donato

3. LaTeX – Prof. Spinozzi Francesco

4. Getting started with R: Environmental Computing – Dr. D'Errico Giuseppe

5. Assessing oxidative stress in biological systems – Prof. Damiani Elisabetta

6. Shot of Science, speakers: "Cristina Maracci 29.11.22 ; " Giulia Lucia 20.12.22" ; " Alessia Pepe 28.02.23" ; " Giorgia Giorgini 18.04.23" ; "Alessandra Gilda Ritacca 09.05.23 " ; "Marta Lombò Alonso 30.05.23 ".

7. In vitro models mimicking human tissues and their cross-talk", Monica Mattioli Belmonte Cima, Dipartimento di Scienze Cliniche e Molecolari, Università Politecnica delle Marche - 13.06.23: 11:30-12:30

List of periods spent abroad

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List of conferences/workshops attended and of contributions eventually presented

1. **TUM Conference** "Researchers together: comparison and sharing of SIB themes between Toscana, Umbria and Marche, 1 December 2022 Perugia, Italy. *Poster presentation:* Luccarini A., Marcheggiani F., Tiano L., Damiani E.: Characterization of new photo-protective agents from *Cyclopia spp.* (Honeybush) as bioactive UV filters for skin protection.

2. CAS SciFinder^{n} Training, 7 February 2023.

3. **The 47th FEBS Congress** "Together in bioscence for a better future". 8-12 July 2023, Tours, France. *Poster presentation*: Luccarini A., Marcheggiani F., Tiano L., Joubert E., De Beer D., Damiani E.: Characterization of the photo-protective ability of Cyclopia spp. (Honeybush) extracts as new UV filters for skin protection.

4. **20th Congress of the European Society for Photobiology,** 26-31 August 2023, Lyon, France. *Poster presentation:* Luccarini A., Marcheggiani F., Tiano L., Joubert E., De Beer D., Damiani E.: Characterization of the photo-protective ability of Cyclopia spp. (Honeybush) extracts as new UV filters for skin protection; Luccarini A., Zuccarotto A., Castellano I., Damiani E.: Insights on the photoprotective potential of marine-inspired thiol compounds.

5. **3rd DISVA Biochemistry, Molecular Biology and Cellular Physiology Summer Retreat,** 31 August- 4 September 2023, Calcinaia sul lago, Sansepolcro, Italy.

6. **Third MaSBiC Symposium** - Advances in Protein Science: Exploring Structure, Function, and Beyond, 20-22 September 2023, Ancona, Italy. *Poster presentation*: Luccarini A., Zuccarotto A., Castellano I., Damiani E.: Insights on the photoprotective potential of marine-inspired thiol compounds.

Part 3. PhD student information on publications

List of publications on international journals

J1. Busto F, Licini C, Luccarini A, Damiani E, Mattioli-Belmonte M, Cometa S, De Giglio E. Oleuropein-Rich Gellan Gum/Alginate Films as Innovative Treatments against Photo-Induced Skin Aging. Molecules. 2023; 28(11):4352. doi: 10.3390/molecules28114352.

J2. Morresi C, Luccarini A, Marcheggiani F, Ferretti G, Damiani E, Bacchetti T. Modulation of paraoxonase-2 in human dermal fibroblasts by UVA-induced oxidative stress: A new potential marker of skin photodamage. Chem Biol Interact. 2023 Sep 15; 384:110702. doi: 10.1016/j.cbi.2023.110702.

J3. In preparation. Luccarini A, Zuccarotto A, Galeazzi R, Morresi C, Masullo M, Damiani E, Castellano I. Insights on the photoprotective potential of marine-inspired thiol compounds.

List of publications on conference proceedings

List of other publications (books, book chapters, patents)

[13.10.23]

Student signature

Alessia Luccarini

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Supervisor signature

Elisabetta Damiani

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