PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

Report Form for PhD student annual evaluation (XXXVII cycles, PON)

Name of PhD student: .....Eleonora Mari.....

Title of PhD research: Green economy: the potential role of the hope plants, from flowers to leaves.

Name of PhD supervisor: .....Maria Grazia Ortore.....
Research lab name: .....Laboratorio di Biofisica Molecolare.....

Mesi in IMPRESA (Italia): .....8.....
Mesi in IMPRESA (Estero, se previsti): .....0.....

Cycle:
[ ] XXXVI
[X] XXXVII

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
[ ] Advanced Instrumentation lab
[ ] Aquarium
[ ] MassSpec lab
[ ] MaSBiC
[ ] Simulation/informatics lab
[ ] Other. Please, indicate: .....

ABSTRACT:
The main idea of this project is to exploit the bioactive compounds extracted from the waste of the hop plant: the leaves, which are not used for commercial aims and are often burned after the harvest. It is hence crucial to understand how to optimize the extraction of bioactive compounds and to make possible their use in different kind of products. The biomass of the hop plant contains several principles that have been shown to have antibacterial and antiviral properties, and other compounds able to interfere with the amyloid fibrillation process, the cause of the most important neurodegenerative diseases.
Hop biomass has hence several possible applications ranging from pharmaceutical up to nutraceutical industries, because hop waste can be transformed into natural additives for food preservation and for pharmacological aims.

Part 1. Scientific case of the PhD Research

BACKGROUND: This project needs an integrated approach which ranges from biophysics to microbiology. The potential of the hop plant is wide, and an extensive experimental work is required to test its efficiency. Recent studies have shown that hops are rich in bioactive compounds with antibacterial, antioxidant, anti-inflammatory and antiviral properties[1]. Beneficial effects have also recently been demonstrated in Alzheimer's disease (AD)[2], the most common cause of dementia, although the mechanism of action of hops compounds is still far from being understood[3]. Currently the most investigated bioactive compounds are xanthohumol, humulone and lupulon[1].

SCIENTIFIC AIMS: The objectives of this research will be to investigate functional groups present in hops extracts, whose biological activity has not been evaluated. We will monitor the influence of saponins, xanthohumol, α-humulene, and the whole extract on amyloid aggregation and the antimicrobial capacities for future application in the food and health issues. We will investigate human insulin and amyloid beta peptide, proteins involved in human diseases.[4] Because hop extracts are obtained from agricultural waste, the aim is to reuse and develop a circular and green protocol.

WORKPLAN AND RESEARCH ACTIVITIES:

WP 1. Production and analysis of polyphenol content of the hop extract for use in cosmetic products.

Methods. In order to include the hop in cosmetic products, we produced the extracts in two different solvents: glycerin and propylene glycol. The extraction of bioactive compounds from the hop was performed using the previously produced hop powder. To optimize the extract, we tested different concentrations of glycerin (most used in cosmetics) and propylene glycol, at different temperatures, for different times and we analyzed the content of polyphenols with the Folin-Ciocalteu assay. The extraction was performed using a stirrer and a dilution factor of 1:20 (1g of hop's powder in 20 ml of solvent). The suspension was centrifuged (5000 rpm, 10 min) and filtered with a filter paper. The extract is stored at room temperature.

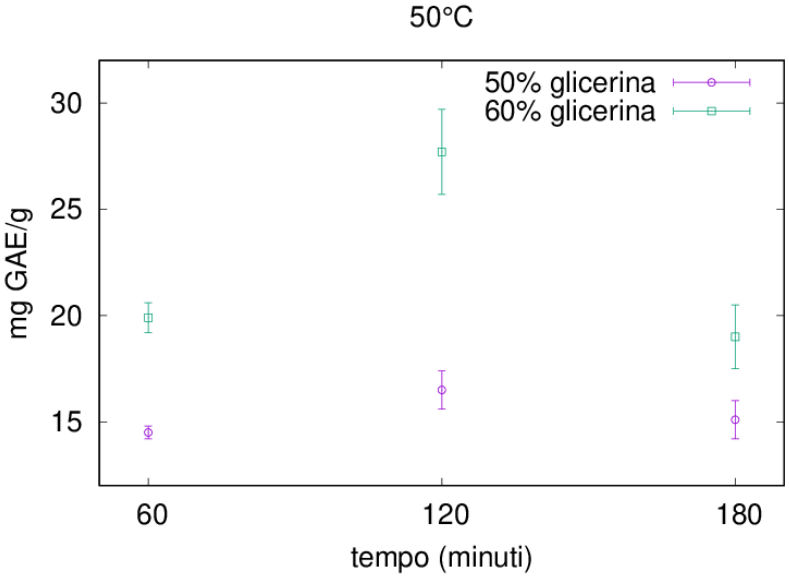

Figure 1. Data obtained for Folin-Ciocalteau assay for glycerin extracts (most used in cosmetics) at 50°C (best temperature condition).

Expected/Obtained Results. Our results shown that the extract with the highest polyphenol content was the one in glycerin 60%, extracted at 50° C for 120 min.

WP 2. Development and test of biocosmetics with hop leaves extract.

Methods. From February to October, I had the opportunity to develop and test biocosmetics with extracts of hop leaves. After becoming familiar with the instrumentation of the Pierpaoli SRL company laboratory, I started to participate in the research and development activity. In order to insert our extracts in cosmetic products, we carried out, in the microbiology laboratory of the company, a microbiological investigation of the extracts to evaluate the possible presence of microbial charge, of mold and of yeast. Experimental results on the microbiological content of our extracts, without any care in their collection for 4 months at ambient temperature, were very promising because there had been no contamination at all. Hence, I took care of the preparation of the bases for the products; we chose two rinse products, a conditioner and a shampoo that differ for chemical composition, structure and chemical-physical features (such as pH, viscosity etc), being the recipients of our extracts. Once I prepared both the bases (for conditioner and for shampoo), we performed the stability tests of the products to evaluate the shelf-life, measuring pH, viscosity, turbidity, and color changes. These measurements have to repeat several times. We prepared 3 samples for each product, for both extracts, and collect them for 3 months. Each sample has been maintained in 3 different conditions: i) at 4 ºC, ii) at room temperature, and iii) at 40 ºC. We checked the parameters at regular intervals.

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Expected/Obtained Results. From the stability tests, the best shelf-life obtained was for products containing glycerin extract, both for conditioner and shampoo. In comparison to products with glycolic extract, they show better appearance and functionality.

WP 3. Study of the effect of saponins (contained in hops) on the amyloid aggregation of human insulin

Methods.

To confirm our previously obtained data (UV-Visible absorption spectroscopy, using Congo Red as amyloid specific probes to monitor the increase of β-structures, Small Angle X-ray Scattering and Dynamic Light Scattering experiments to obtain the overall structural features and size of the aggregates) we performed fluorescence spectroscopy, using Thioflavin T as a specific probe to link to β-structures, and Circular Dichroism experiments, to monitor any secondary structure changes (from α-helix to β-sheets). The amyloid aggregation was performed with commercial human insulin (Sigma Aldrich) at a concentration of 0,5 mg/ml in phosphate buffer 50mM, pH 7.4, and 37°C under stirring.

Expected/Obtained Results. By the fluorescence spectroscopy experiment of human insulin with Thioflavin T, we expect to obtain an increase in fluorescence when β-structures increase. in absence of saponins, the formation of β-structures is observed since the fluorescence signal increases during 48h. On the other side, in presence of saponins the signal remains constant, suggesting no structural change, neither aggregation. Circular Dichroism experiments show that the spectrum obtained after 48h, in presence of saponins, remains the same one corresponding to native protein, while in absence of saponins the spectrum obtained is characteristic for the β-structures. Our experimental data confirm that saponins interfere with the amyloid aggregation by inhibiting the formation of insulin fibrils, maintaining the secondary structures of the protein.

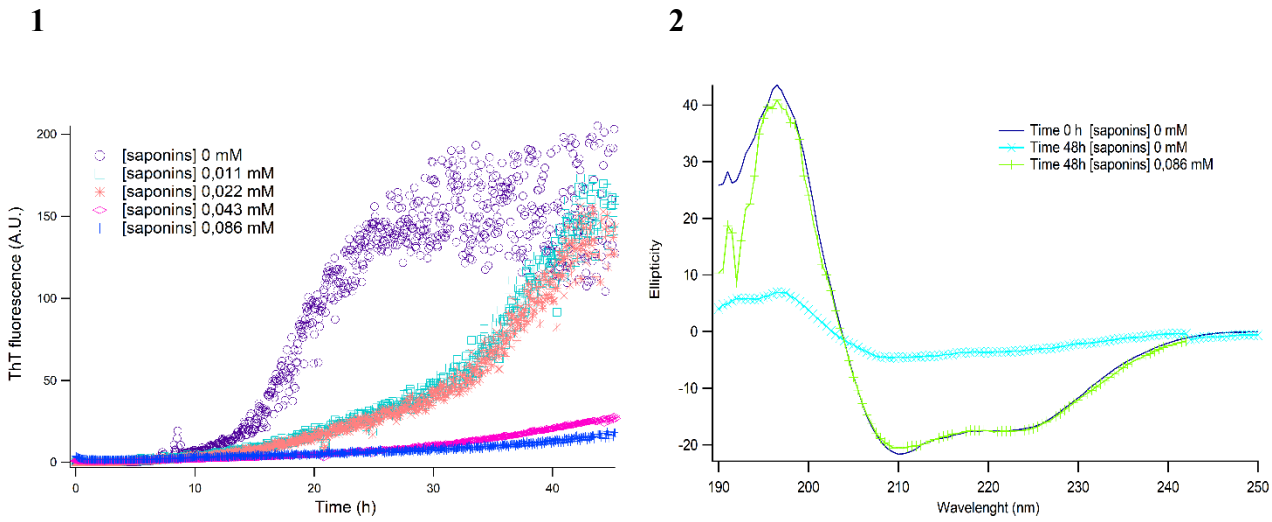

Figure 2. **[1] Fluorescence Spectroscopy** and **[2] Circular Dichroism** data obtained for human insulin in phosphate buffer 50 mM, pH 7,4 at 37° C in gentle agitation, in absence and presence of saponins (molar ratio insulin-saponins 1:1), as a function of time

We will conduct further studies with other bioactive compounds of hop biomass (xantumol, α-humulene) to compare the effects on the amyloid aggregation, and to evidence possiblesynergies between different compounds.

WP 4. Study of the effect of hop extract, produced from plant waste, on the amyloid aggregation of human insulin.

Methods. The experimental conditions for insulin aggregation were the same investigated in WP2. We monitored the increase of β-structures, as a function of time, in presence and in absence of the hop extract, by fluorescence spectroscopy, as in WP2. We performed Circular Dichroism experiments, to monitor any secondary structure changes, as in WP2.

Expected/Obtained Results.

Our experimental results show that hop extract interfere with the amyloid aggregation, proving that these induce a high inhibition on the formation of insulin fibrils, maintaining the secondary structures of the protein.

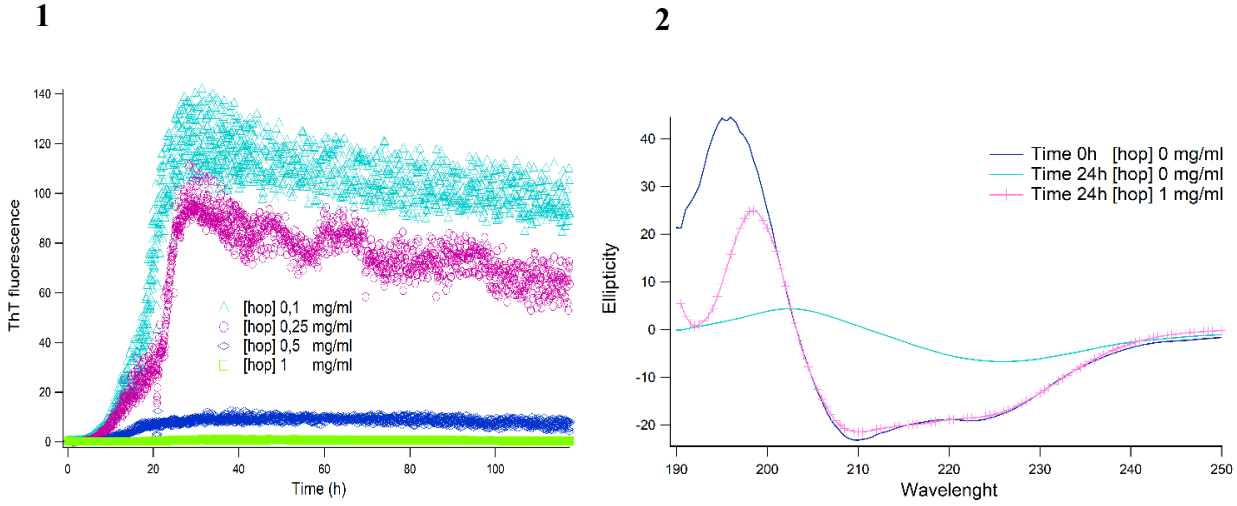

Figure 3. **[1] Fluorescence Spectroscopy** and **[2] Circular Dichroism** data obtained for human insulin in phosphate buffer 50 mM, pH 7,4 at 37˚ C in gentle agitation, in absence and presence of hop extract, as a function of time.

- REFERENCES

1. Bogdanova K. et al. (2018). Antibiofilm activity of bioactive hop compounds humulone, lupulone and xanthohumol toward susceptible and resistant staphylococci. *Research in microbiology*, *169*(3), 127–134. https://doi.org/10.1016/j.resmic.2017.12.005Huang X et al. (2018) The Prenylflavonoid Xanthohumol Reduces Alzheimer-Like Changes and ModulatesMultiple Pathogenic Molecular Pathways in the Neuro2a/APP_{swe} Cell Model of AD. *Front. Pharmacol.* 9:199.doi: 10.3389/fphar.2018.00199

2. Palmioli, A. et al. (2022). Alzheimer's Disease Prevention through Natural Compounds: Cell-Free*, In Vitro*, and *In Vivo* Dissection of Hop (*Humulus lupulus* L.) Multitarget Activity. *ACS chemical neuroscience*, 10.1021/acschemneuro.2c00444. Advance online publication. https://doi.org/10.1021/acschemneuro.2c00444

3. Chiti, F., & Dobson, C. M. (2006). Protein misfolding, functional amyloid, and human disease. *Annual review of biochemistry*, *75*, 333–366. https://doi.org/10.1146/annurev.biochem.75.101304.123901

4. Knez Hrnčič, M., Španinger, E., Košir, I. J., Knez, Ž., & Bren, U. (2019). Hop Compounds: Extraction Techniques, Chemical Analyses, Antioxidative, Antimicrobial, and Anticarcinogenic Effects. *Nutrients*, *11*(2), 257. https://doi.org/10.3390/nu11020257

5. Mari E.et al. *Trehalose Effect on The Aggregation of Model Proteins into Amyloid Fibrils* Life 2020**,** 10,60; doi: 10.3390/life10050060

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

List of attended courses/seminars/schools

Schools:

From 16 to 22 October participation at the International School of Biophysics "Antonio Borsellino", 48th Course: memos for biophysics into the future: lightness, quickness, exactitude, visibility, multiplicity and consistency, in Erice (TP).

Seminars:

1. 13/06/23 "In vitro models mimicking human tissues and their cross-talk", Monica Mattioli Belmonte Cima, Dipartimento di Scienze Cliniche e Molecolari, Università Politecnica delle Marche
2. 13/06/23 'Speech Gym with your colleague Giulia', Dott.ssa Giulia Lucia
3. 14/06/23 'Facilities at DISVA: FTIR/Raman (Valentina Notarstefano), Imaging cellulare (Fabio Marcheggiani), AFM/DLS (Paolo Moretti), Microscopia Confocale (Andrea Frontini), MASBIC (Alice Romagnoli), Aquarium (Alessio Zenobi), MassSpectLab (Marta Di Carlo), Boa oceanografica Fortunae (Pierpaolo Falco e Francesco Memmola) e Laboratori mobili Actea e Mytilus (Fabrizio Torsani)'.
4. 16/06/23 'Mechanism of nano and microplastics capture by jellyfish mucin and its potential as a sustainable

water treatment technology', Isam Sabbah, Prof. Ephraim Katzir Department of Biotech- nology Engineering, BRAUDE College of Engineering, Karmiel, Israel

5. 25/10/23 'Biotechnological applications of microalgae Chlamydomonas Reinhardtii', Dott.ssa Amina Antonacci

List of periods spent abroad

1. None

List of conferences/workshops attended and of contributions eventually presented

1. From 19 to 20 April 2023 participation at the Biophysics@Rome2023, in Rome and poster presentation 'Effect of the hop extract and of bioactive compounds from hop plant on the aggregation of human insulin in amyloid aggregation'.

2. From 4 to 8 September 2023 participation at the European Physics Conference in Condensed Matter Division CMD30 (FisMat), in Milan (MI) and poster presentation 'Hop extract can influence amyloid aggregation: focus on human insulin'.

3. From 20 to 22 September 2023 participation at the Third DiSVA-MaSBiC Annual Symposium - Protein Structure and Function in Biology, Medicine and Nanotechnology, in Ancona (AN) and poster presentation 'Hop extract from waste: can hop and it's bioactive compounds influence amyloid aggregation?'.

Part 3. PhD student information on publications

If not yet published, please indicate the publication status (submitted, accepted, in preparation...)

List of publications on international journals

Giulia Sabbatini, Eleonora Mari, Maria Grazia Ortore, Giovanna Mobbili et al. "A multi-disciplinary investigation on the perspectives of hop by-products utilization: progress towards circular economy" *in submission*

Mari E., Galeazzi R., Ortore M.G. "Effect of Saponins on the aggregation of Human Insulin in amyloid fibrils" *in preparation.*

List of publications on conference proceedings

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

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08/11/23

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Supervisor signature