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September 1-5, 2019
Peniche / Portugal

Scientific Program

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PC157: Anti-Aging Activity of *Lobophora Variegata* Ethanolic and Methanolic Extracts and Their Fractions

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Seaweed have promising applications within food, cosmetic and health industries, which led to an increased interest in studying these organisms.[1] In several coastal areas, thousands of tons of macroalgae are cast on beaches and shorelines and it would be very interesting if this biomass could be managed, allowing the extraction of added-value compounds. In this context, polar extracts (methanol and ethanol) of a macroalgal beach cast sample mainly composed of *Lobophora 263orphyrin* were prepared and the anti-aging and antioxidant activities were evaluated. The preliminary results showed interesting results, and thus these crude extracts were then fractionated sequentially by their solubility in dichloromethane, acetone and ethyl acetate, resulting in 4 semi-pure fractions each, which were also tested. Fractions A1.1.1 and A1.2.3 were very good tyrosinase inhibitors (IC_{50} = 37.87 and 24.01 μg/mL, respectively) and fractions A1.1.2 and A1.2.2 presented very good inhibition of elastase (IC_{50} = 44.76 and 20.86 μg/mL, respectively). However, none of the fractions was active against collagenase. These results show that further purifications of these fractions can lead to the isolation of bioactive added-value compounds.

Acknowledgements: Study and G. Rosa's grant financed by: MACBIOBLUE project (MAC/1.1b/086, Interreg Mac 2014–2020 and DRCT); FCT – Fundação para a Ciência e a Tecnologia, the European Union, QREN, FEDER, COMPETE, by funding the cE3c centre (FCT Unit funding (Ref. UID/BIA/00329/2013, 2015 – 2018) and UID/BIA/00329/2019) and the QOPNA research unit (project FCT UID/QUI/00062/2019).

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ANTI-AGING ACTIVITY OF *Lobophora variegata* ETHANOLIC AND METHANOLIC EXTRACTS AND THEIR FRACTIONS

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Introduction

Macroalgae have promising applications within the food, cosmetic and health industries, which led to an increased interest in studying these organisms. In several coastal areas, thousands of tons of macroalgae are cast on beaches and shorelines, and it would be relevant if this biomass could be managed, allowing the discovery of added-value applications. *Lobophora variegata* is a small brown macroalga with a wide distribution in temperate and tropical seas. Although *L. variegata* is found in large quantities in Macaronesia, there are no commercial applications for this alga to date.

Delaying the effects of aging is a strong motivation to look for products that can inhibit the action of various enzymes that contribute to outward signs of skin aging (Fig 1).[1] Tyrosinase inhibitors can be used as effective skin lightening agents.[1] Elastase is an enzyme that degrades elastin, an essential protein responsible for skin elasticity, while collagen, responsible for the firmness of the skin, is degraded by collagenase. Inhibitors of any of these enzymes will be useful in treating wrinkles and sagging skin.[1,2] On the other hand, exposure to UV radiation lead to the formation of free radicals, causing damage and skin aging.[2] Antioxidant activity is thus vital for neutralizing these free radicals and consequently reducing the damage caused.

The present study, inserted in the context of project MACBIOBLUE (INTERREG MAC/1.1b/086), aims to evaluate the potential of *L. variegata* to incorporate products that contribute to healthy aging. In this context, the polar extracts (methanol and ethanol) of a macroalgal beach-cast sample of mainly *L. variegata* and their fractions were prepared, and the anti-aging and antioxidant activities were evaluated.

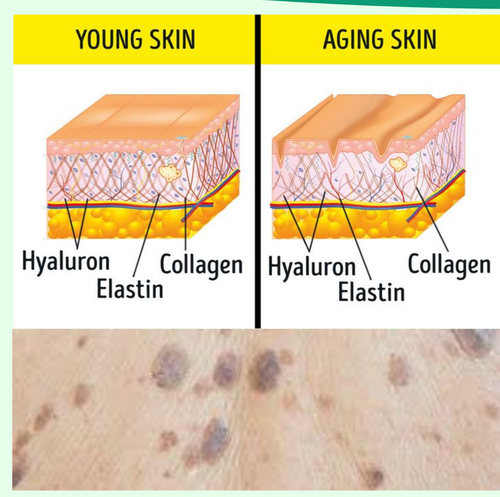

Fig. 1. Skin changes with aging.

Methods and Material

1- Crude Extracts and Fractions Preparation

Two portions of A1 (100% *L. variegata*) harvested in the Gran Canaria coast, were separately extracted with methanol and ethanol (10 g in 300 mL) using an ultrasonic bath (1h 30 min) at room temperature. After centrifugation, the supernatant from each extraction was recovered and the solvent evaporated to yield 1.87 g (A1.1) and 1.7 g (A1.2) of crude methanol and ethanol extracts, respectively.

Each extract was fractioned by its solubility in dichloromethane (fractions A1.1.1, 712 mg, and A1.2.1, 473 mg), acetone (fractions A1.1.2, 195 mg, and A1.2.2, 203 mg), ethyl acetate (fractions A1.1.3, 125 mg, and A1.2.3, 82 mg). The remain insoluble material were the fractions A1.1.4 (718 mg) and A1.2.4 (718 mg).

2- Biological Activities Assessment

Anti-tyrosinase, anti-elastase, anti-collagenase and antioxidant activities were performed by adapting the protocols described in Manosroi et al.,[3] Ndlovu et al.,[4] Thring et al.[5] and Blois,[6] respectively. All these methods are based on spectrophotometric measurements (Fig. 2).

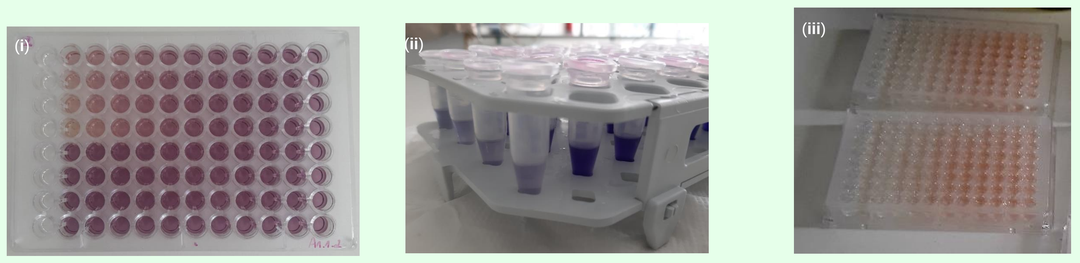

Fig. 2: Some details of the determination of the antioxidant (**i**), anti-colagenase (**ii**) and anti-tyrosinase (**iii**) activities of the methanol and ethanol extracts of *L. variegata* and their fractions.

Results and Discussion

Crude extracts, as well as all fractions obtained from them, were evaluated for their anti-tyrosinase, anti-elastase, anti-collagenase and antioxidant activities, and the following positive controls were used: Kojic Acid (**A**), N-Methoxy-succinyl-Ala-Ala-Pro-chloromethyl-S (**B**), EDTA (**C**) and Trolox (**D**), respectively. The results obtained are presented in Table 1.

Table 1: Anti-aging activities of *L. variegata* crude extracts (A1.1 and A1.2), methanolic extract fractions (A.1.1.1 to A.1.1.4), ethanolic extract fractions (A.1.2.1 to A.1.2.4), and reference compounds.

	Anti-tyrosinase	Anti-elastase	Anti-collagenase		Antioxidant	
	IC_{50} (µg/mL)	IC_{50} (µg/mL)	IC_{50} (µg/mL)	% inhibition*	IC_{50} (µg/mL)	% RSA**
A1.1	66.0 ± 10.3	>250	195.6 ± 6.1	61.5 ± 1.1	156.2 ± 3.1	69.4 ± 1.5
A1.1.1	37.9 ± 1.2	173.3 ± 5.4	>250	1.6 ± 0.3	213.0 ± 0.1	52.8 ± 0.6
A1.1.2	69.4 ± 1.2	44.8 ± 2.1	>250	6.8 ± 1.9	225.0 ± 15.1	52.1 ± 2.2
A1.1.3	>250	>250	>250	6.3 ± 1.3	>250	20.6 ± 0.9
A1.1.4	167.0 ± 8.2	228.8 ± 10.7	>250	13.3 ± 0.6	>250	23.8 ± 1.0
A1.2	59.1 ± 6.9	>250	193.2 ± 15.0	55.9 ± 3.7	81.6 ± 2.1	72.8 ± 0.6
A1.2.1	97.7 ± 0.52	61.3 ± 0.93	>250	13.6 ± 1.92	>250	23.8 ± 1.02
A1.2.2	>250	20.9 ± 0.39	>250	35.9 ± 0.92	223.3 ± 2.61	53.0 ± 1.31
A1.2.3	24.1 ± 0.24	>250	>250	13.4 ± 1.80	>250	34.5 ± 1.44
A1.2.4	>250	>250	>250	12.4 ± 2.79	>250	20.7 ± 0.45
A	1.82 ± 0.13					
B		0.13 ± 0.002				
C			59.3 ± 3.0	ND		
D					7.25 ± 0.09	89.71±0.50

***** at a maximum concentration of 250 µg/mL. **RSA – Radical Scavenging Activity at 250 250 µg/mL. ND – Not Determined

❖ The results of table 1 show that the fractionation carried out resulted in fractions richer in compounds with anti-tyrosinase and anti-elastase activity, with a significant increase in activity. Chemical analysis of these two fractions is underway and will identify the compounds responsible for the anti-elastase activity displayed.

❖ On the other hand, concerning anti-collagenase and antioxidant activities, fractionation led to a loss of activity. In fact, although much less active than the reference compounds (EDTA and trolox), crude extracts are more active than the corresponding fractions.

❖ Regarding anti-tyrosinase activity, the fraction that showed the highest activity was the ethyl acetate fraction from the ethanol extract (**A1.2.3**), followed by the dichloromethane fraction from the methanol extract (**A1.1.1**), although both were much less efficient in tyrosinase inhibition than the reference compound, kojic acid (**A**).

❖ In the anti-elastase activity, the acetone fraction of the ethanol extract (**A1.2.2**) shows the highest activity followed by the acetone fraction of the methanol extract (**A1.1.2**), and in both cases, the IC_{50} values recorded are much lower than those shown by crude extracts, which is the most interesting result of this work. The chemical analysis of these two fractions is underway and will identify the compounds responsible for the displayed anti-elastase activity.

❖ In conclusion, *L. variegata* algae has potential for application in products with anti-aging activity. Notably, the dichloromethane and ethyl acetate fractions of the methanol and ethanol extracts, respectively, can be used as sources of skin-blemish-acting compounds, and the acetone fractions of both extracts as a source of agents that promote elasticity of the skin.

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Acknowledgements:

Study financed by: MACBIOBLUE project (MAC/1.1b/086, Interreg Mac 2014–2020 and DRCT); FCT – Fundação para a Ciência e a Tecnologia, the European Union, QREN, FEDER, COMPETE, by funding the cE3c centre (UID/BIA/00329/2013, 2015 - 2018) and UID/BIA/00329/2019) and the QOPNA research unit (project FCT UID/QUI/00062/2019).