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P65 - PRELIMINARY INVESTIGATIONS ON GRACILARIA GRACILIS CULTIVATION TECHNIQUES AND EXTRACTION OF BIOACTIVE COMPOUNDS WITH ANTIOXIDANT ACTIVITY

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It is know that macro algae can be a valuable source of food and industrial raw materials, such as natural polysaccharides. Furthermore seaweeds are the most promising sources of primary and secondary bioactive metabolites and represent about 9% of biomedical compounds obtained from the sea.
Antioxidants (carotenoids, polyphenols etc.) fatty acids, amino acids, polysaccharides etc., obtained from algae, are useful for many applications, such as nutrition, pharmacology and cosmetics.
These bioactive compounds belong to different classes of chemicals; thus, they can be obtained by several extraction techniques. The toxicity of the solvents used, the degradation of the compounds and the selectivity of the process are major constrains that must be considered in an industrial extraction process. As the bioactive industry is constantly seeking innovation, processes that can increase yield, the productivity, and selectivity are being considered. The Supercritical Fluid Extraction (SFE) is a technique, that allow to modify easily the characteristics of the extracted product, changing the process parameters such as temperature, pressure and co-solvent, ensuring very high yields (1).
Among cultivated algae either on small or large scale, species of the genus Gracilaria have been utilized in several countries, mainly India, Indonesia, Japan. In Italy there are only a few experimental evidences in brackish environments. The commercial interest for Gracilaria derives from the fact that the thallus of many species represent the raw material for the extraction of the agar (2). Agar is used as gelling agent in food and pharmaceutical industry and has an high commercial value.
In this study, we are approaching the experimental techniques of cultivation of Gracilaria gracilis, in the Capo Peloro lagoon (Eastern Sicily; 38°15’57” N; 15° 37’ 50” E), and the extraction of bioactive compounds from cultivated and wild specimens. Square frames (90 cm×90 cm) were used to provide the cultivation structure (3). Each raft held 8 parallel lines of polypropylene rope (3 mm) where vegetative fragments of thalli were inserted (Figure 1). The raft was positioned at about 50 cm from the surface.

Figure 1 – Square frame utilized for G. gracilis cultivation trial

Wild samples of Gracilaria were collected monthly from Margi Canal (the connecting channel between Ganzirri and Faro Lakes). During sampling main physical-chemical water parameters were monitored.
Samples of dry Gracilaria, wild and cultivated, were extracted by ethanol 96%, absolute methanol and supercritical CO2, SFE (4, 5).

Gracilaria extracts were utilized to determine: phenolic content by Folin-Ciocalteu reagent and expressed as gallic acid equivalents (GAE); DPPH radical scavenging assay to evaluate antioxidant activity of 1% extracts and reducing power, as potential antioxidant indicator (4).
Cultivation technique used allowed us to easy manipulate algae. During cultivation trial, although in the short term, it was possible to highlight the influence of environmental factors on the growth of algae. It was found a high mortality when the water temperature began to rise, while, regarding salinity, the algae has been shown to survive for up to salinity of 34‰ .
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Extract	Total antioxidant power (% inib DPPH)	Reducing power	Total polyphenols (mg GAE)
EE	**46**	**0.1**	**60**
ME	**51**	**0.01**	**30**
SE	**64**	**0.6**	**370**

The obtained results showed a significant effect of the method of preparation on the antioxidant power exhibited by the extracts (table 1).
The total antioxidant power has showed the higher value of radical inhibition (64%) for the SFE extract (SE), respect to the methanol (ME) (51%) and ethanol extract (EE) (46%) (table 1).
The total reducing power resulted also higher in the SE (0.5) respect to the ME (0.01) and EE (0.1) (table 1). The total polyphenols contents showed the significant higher value in the extract obtained by SFE (368 mg GAE), compared to ME (30 mg GAE) and EE (60 GAE).
The obtained results suggest that G. Gracilis can be easily cultivated and that it is a potential source of antioxidants; that phenolic compounds might be major contributors to the antioxidant activities and that SFE seems to be the most effective techniques in improve the yield of polyphenols.
Our preliminary results appear useful for further research aiming to isolate and identify the specific phenolic compounds responsible for the antioxidant activity of G. gracilis and underline the importance to select and standardize the extractions methods in order to gain the best yield and to adequately transfer the protocol from laboratory to the industry.
Keywords: Gracilaria; seaweed; biological activity; natural product; antioxidant; SFE.

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