

## ***In vitro* effects of extracts from leaves and rhizomes of *P.oceanica* on HepG2 tumor cells**

Abruscato G.\*, Chiarelli R., Lazzara V., Punginelli D., Mauro M., Di Stefano V., Arizza V., Vizzini A., Vazzana M., Luparello C.

Department STEBICEF, University of Palermo, Viale delle Scienze Bld.16, I-90128 Palermo (Italy)

\* Presenting author email: [giulia.abruscato@unipa.it](mailto:giulia.abruscato@unipa.it)

Bioactive compounds produced by aquatic species exhibit a wide range of therapeutic effects in humans and represent promising prevention and/or treatment agents and beneficial supplements for the formulation of functional food and food-packaging material.<sup>1-2</sup> In order to identify novel potential anti-tumoral substances, aqueous extracts from green (GLE) and beached leaves (BLE) and rhizomes (RE) of the marine seagrass *P.oceanica* were tested on HepG2 hepatocarcinoma (HC) cells to study cell viability/proliferation, cell cycle, apoptosis and autophagy modulation, mitochondrial function and redox state.<sup>3</sup> GLE and RE, but not BLE that was not tested further, affected cell viability in a dose-response manner and the IC<sub>50</sub> at 24h was calculated and used in the subsequent assays. Cell cycle impairment and the accumulation of Annexin-V<sup>+</sup>/PI<sup>+</sup> cells at early times of exposure indicated the apoptosis-promoting effect of both extracts, as also proven by the detection of a panel of activated caspases. The intracellular accumulation of acidic vesicular organelles, hallmarks of autophagy, decreased after both treatments, more drastically after exposure to RE which also induced the loss of mitochondrial transmembrane potential. Notably, viability inhibition was not reverted by co-treatment of RE with the autophagy-stimulator rapamycin, confirming a more extensive cell damage than mere autophagy inhibition. Real time-PCR and Western blot analyses were also performed to check the expression levels of genes and the accumulation of proteins related to the apoptotic and autophagic processes upon treatments. Moreover, only GLE induced the steady downregulation of intracellular ROS. Consistent with a potential suppression of HC metastatic attitude, treatments with both extracts also induced an early block of cell motility in wound healing assays. Overall, the results obtained suggest the potential and diversified anti-HC ability of extracts from *P.oceanica* which appears to be stronger for RE than GLE and merits further investigation to identify the substance(s) responsible for the cytotoxic effect and to open new interesting scenarios for future biomedical and nutraceutical applications.

### **References**

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