

One of the main objectives of chemistry of natural compounds is the identification of biologically active secondary metabolites such as terpenoids, flavonoids, alkaloids, polyphenols, sterols, unsaturated fatty acids, found in vegetable matrices.

Traditionally, the natural sources investigated in our laboratory were mostly plants, resins and essential oils, in order to assess the pharmacological activities of isolated chemical constituents.

The plant matrices are subjected to extraction procedures (liquid-liquid extraction, steam distillation, soxhlet and SPE) spectrophotometric analysis (UV, IR, NMR), chromatographic treatments (TLC, flash column chromatography, HPLC and GC).

The conducted phytochemical investigations have included: oleogum resins produced by different types of trees of the genus *Boswellia spp.* and their essential oils, *manna* produced from cultivars of *Fraxinus angustifolia* and *Fraxinus ornus*, oranges and grapefruits of the species *Citrus sinensis* (L) Osb., *Citrus aurantium* subsp. *Myrtifolia*, *Citrus paradisi*, aerial parts of *Athamanta sicula* L. and *Euphorbia bivonae* Steudel, resins and aerial parts of *Dracaena draco* L.. The chemical constituents isolated from plant material were tested for their antimicrobial activity, antioxidant, antiproliferative and antibiofilm properties.

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Cadmium induces autophagy during development of *Paracentrotus lividus* embryos

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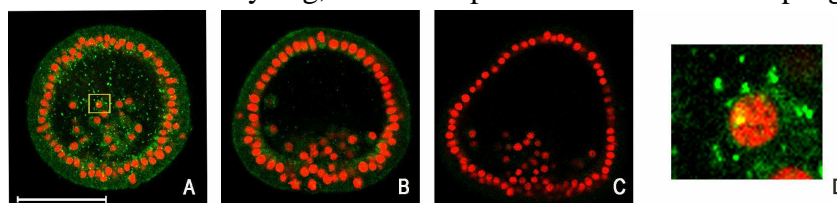
P. lividus embryos adopt different defense strategies against cadmium stress such as synthesis of hsp's and/or activation of apoptosis [Roccheri et al., 2004; Agnello et al., 2007; Agnello and Roccheri 2010]. Here we show that this model system adopts autophagy as an additional strategy to safeguard the developmental program and we show an interesting relationship with apoptosis. It is known that autophagy can be a survival mechanism, but also a device of PCD-II. The use of several investigation methods are suitable for autophagy detection (Kelekar, 2005).

We found that in *P.lividus* embryos autophagic processes occur, at basal levels during physiological development and at greater levels after 1mM CdCl₂ treatment.

By Acridine Orange vital staining on whole embryos and Confocal Laser Scanning Microscopy (CLSM) we detected the acidic vesicular organelles (AVOs) or autophagolysosomes rate. In order to confirm this data we employed an antibody against LC3 protein, a specific marker of autophagy, both through Western blotting and immunofluorescence/CLSM analysis.

Concomitantly bafilomycin A1 has been used as a late inhibitor of autophagy.

Furthermore we have detect the temporal and functional autophagy/apoptosis relationship analyzing, in the presence of the autophagy



Immunofluorescence analysis of LC3 protein in whole embryos, after 18 hours of development. Equatorial optical sections captured by CLSM. In green LC3 protein, in red nuclei stained with propidium iodide. A) Cadmium treated embryo B); Control embryo; C) Negative control; D) Enlargement of a particular of A. Bar=50µm