

IECN  
2020

# The 1st International Electronic Conference on Nutrients Nutritional and Microbiota Effects on Chronic Disease

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## Biopotential of sea cucumbers (Echinodermata) and tunicates (Chordata) from the Western coast of Portugal for the prevention and treatment of chronic illnesses

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# Non-communicable diseases

- Cancer
- Diabetes
- Arthritis
- Heart diseases
- Fibromyalgia
- Hypertension
- Osteoporosis/osteopenia
- Autoimmune diseases
- Neurodegenerative diseases...

OXIDATIVE  
STRESS

Chronic  
Diseases

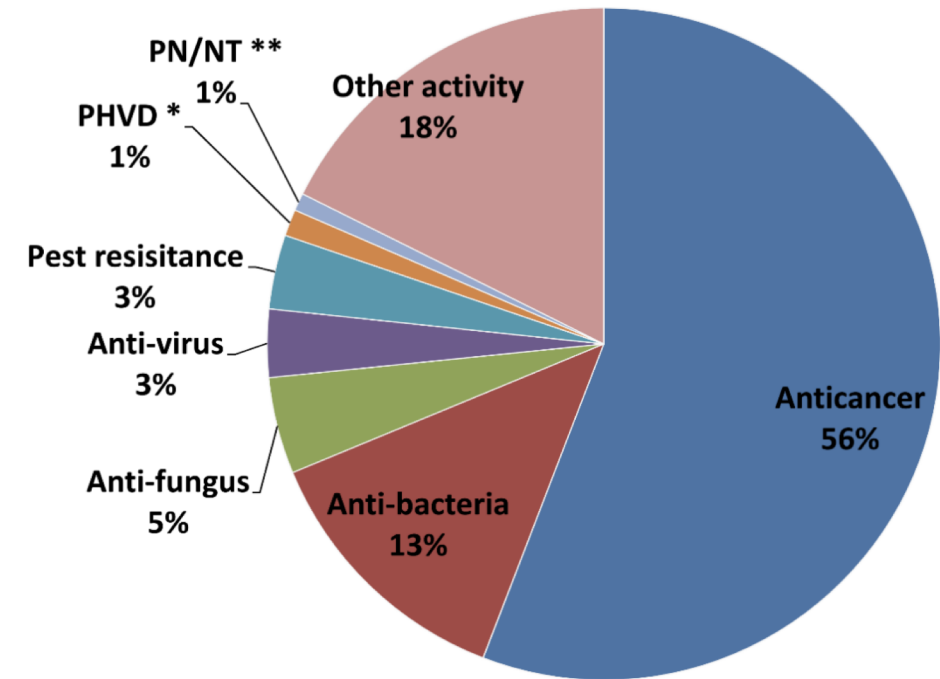
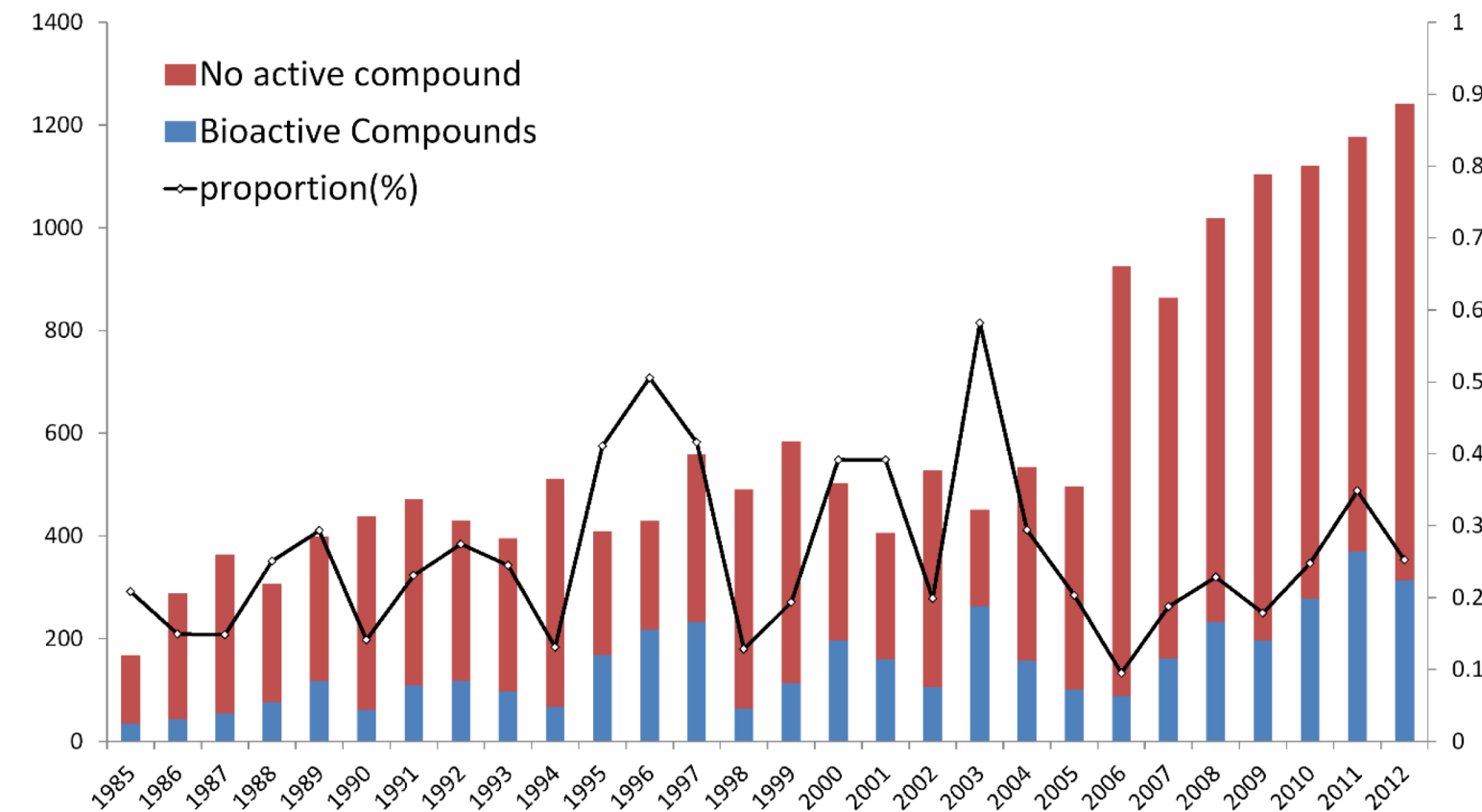
INFLAMMATION



...Nutraceuticals are important tools for the prevention

# Marine Derived Natural Compounds

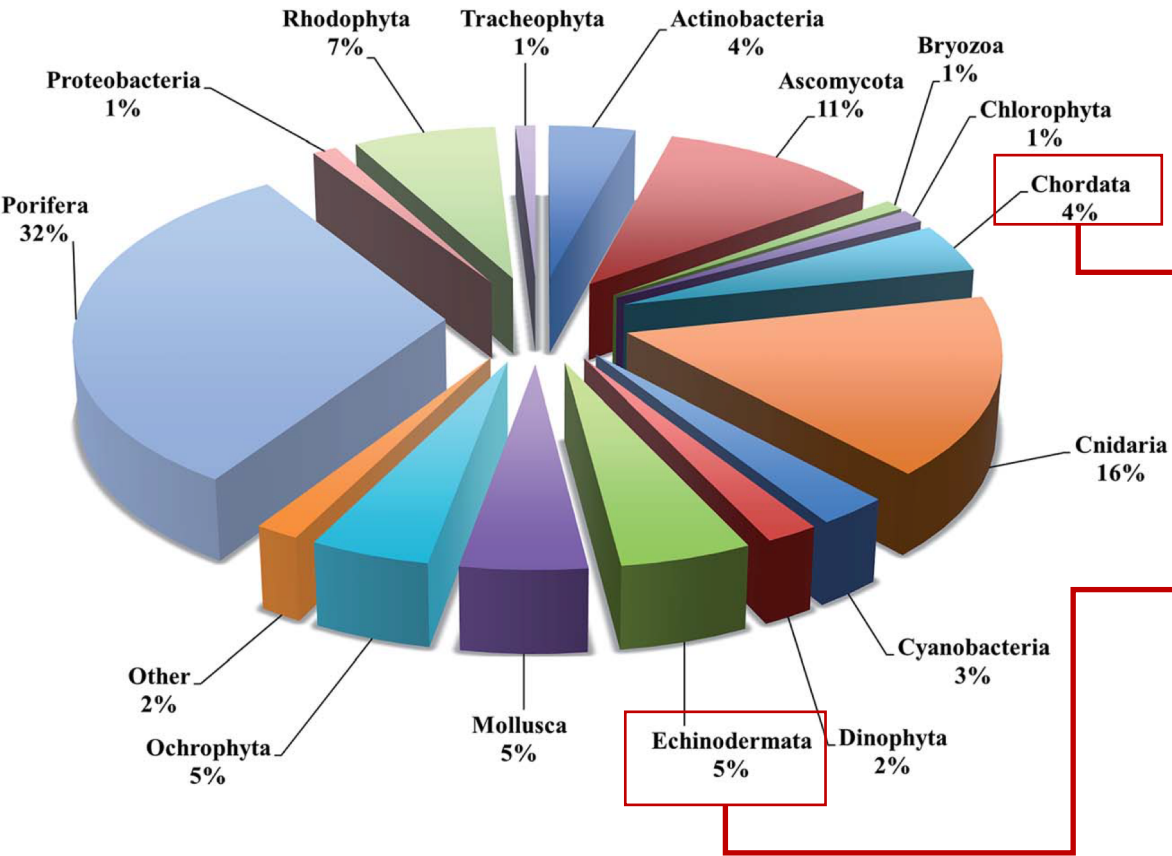
- Rising interest from the pharmaceutical, cosmetic and food supplements industries
- Enormous pool of biodiversity and their associated metabolic diversity
- Potentially infinite source of bioactive compounds



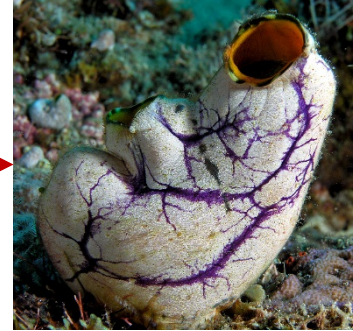
Hu et al. *Marine drugs* 13.1 (2015): 202-221

# Sea cucumbers and Ascidians

Marine invertebrates are major sources of novel bioactive compounds and the taxonomic diversity of these prolific groups is wide.



## Ascidiacea



- Highly diverse group
- Several bioactive compounds isolated from sea squirts and associated microbiome

## Holothuroidea



- Used in traditional medicine by communities of South-West Asia
- Excellent profile of vitamins and nutrients
- Growing market

Blunt et al. (2017). *Natural product reports*, 34(3), 235-294.

# Extracts production

## 1. Solvent extraction

15h  
300 rpm



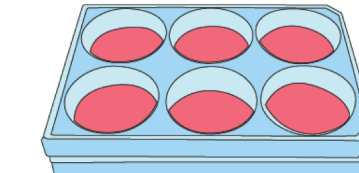
Solvents: water, ethanol, ethyl acetate  
Solvent-Biomass ratio = 30 ml : 1g

## 2. Centrifugation (x3)

5'  
2500 rpm



No Evaporation  
(concentration 1:1)



Anti-oxidant potential  
Antiinflammatory assay

## 3. Concentration



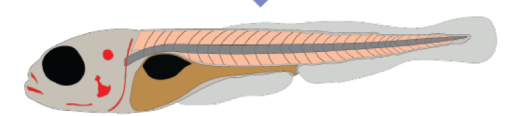
## 4. Evaporation



weak flow of N<sub>2</sub>



Resuspension in  
water, ethanol and DMSO

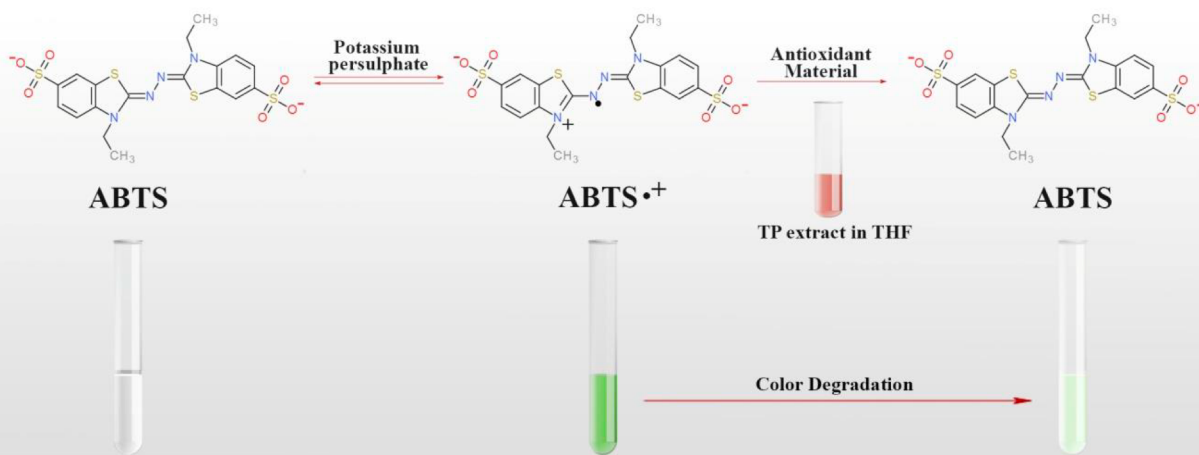


Osteogenic potential

# In vitro Anti-oxidant and Anti-inflammatory potentials

## Antioxidant potential

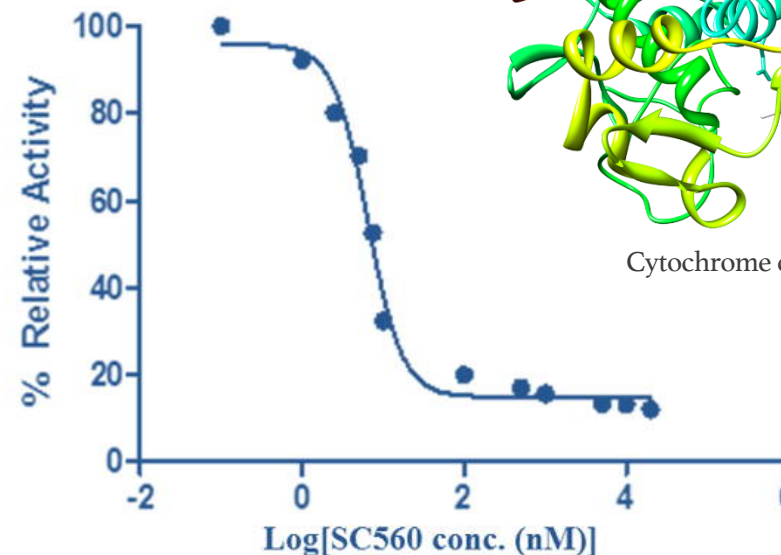
- DPPH · method
- ABTS<sup>+</sup> method
- FRAP method
- Polyphenols quantification



From Mehmet Üstündaş et al. (2018). *Anadolu University of Sciences & Technology-A: Applied Sciences & Engineering*, 19(2).



Cytochrome c at English Wikipedia / CC BY-SA



## Anti-Inflammatory potential

- Cyclooxygenase 2 (COX<sub>2</sub>) Inhibitor Assay

# In vivo Osteogenic activity



Zebrafish  
(Danio rerio)

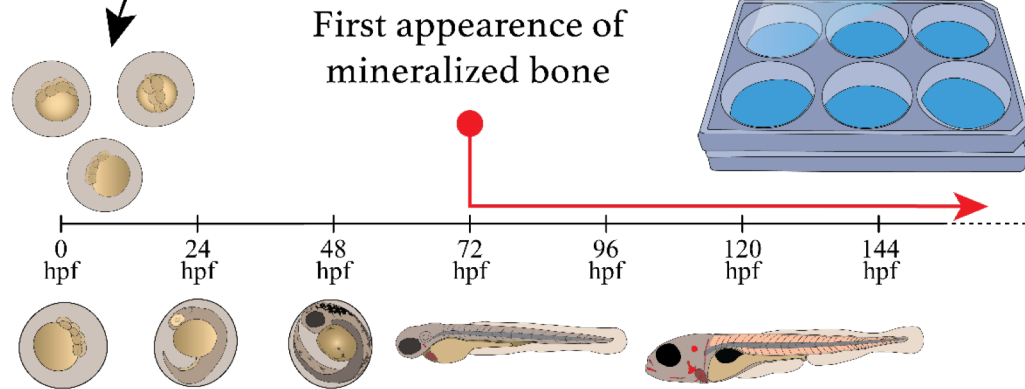
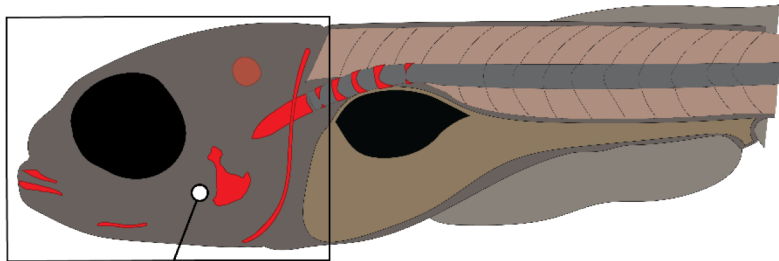
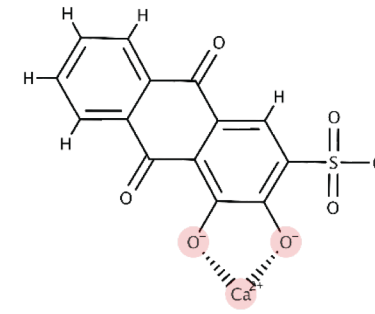


Image analysis:  
ZFBONE, Tarasco et al. 2020

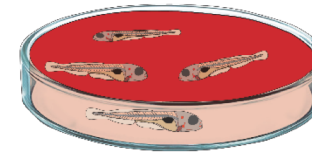


Operculum area (OpA) / Head area (HA)

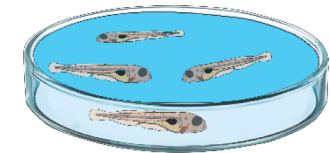
6-Well Plate  
- 15 larvae/well  
- 10 ml /well  
- medium renewal  
70% daily



- Alizarin Red S staining  
(calcium binding dye)  
- Rinse (2x) in fish water



0.03% - 20 min.

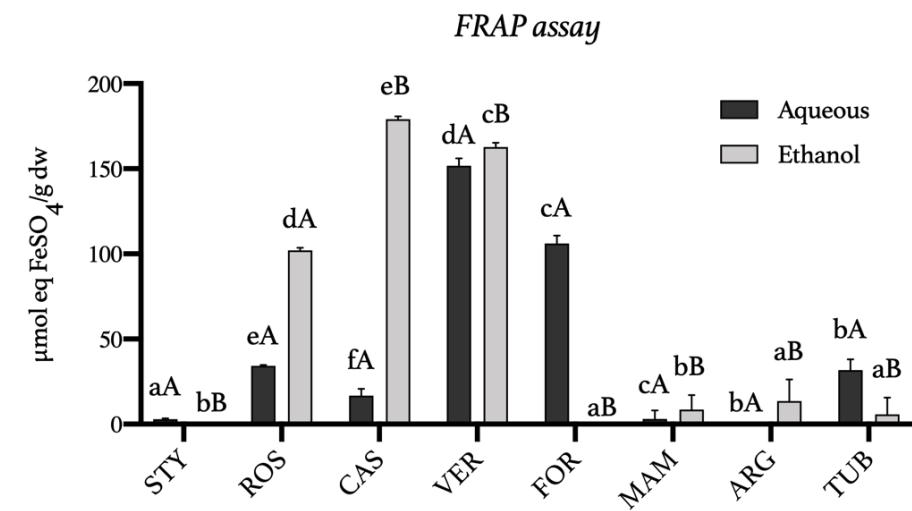
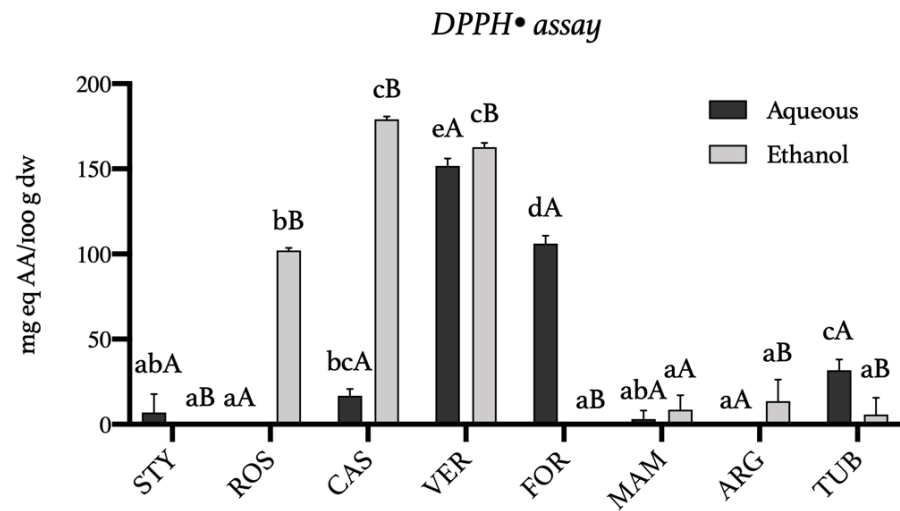
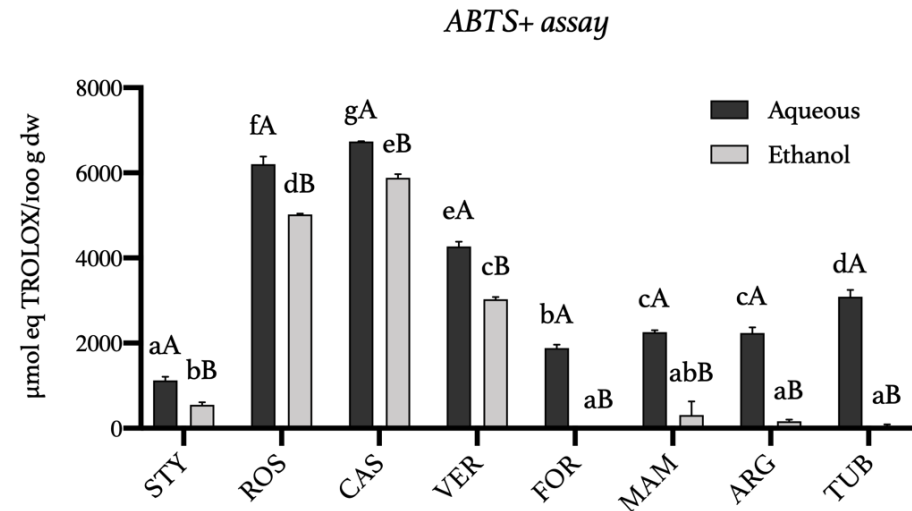
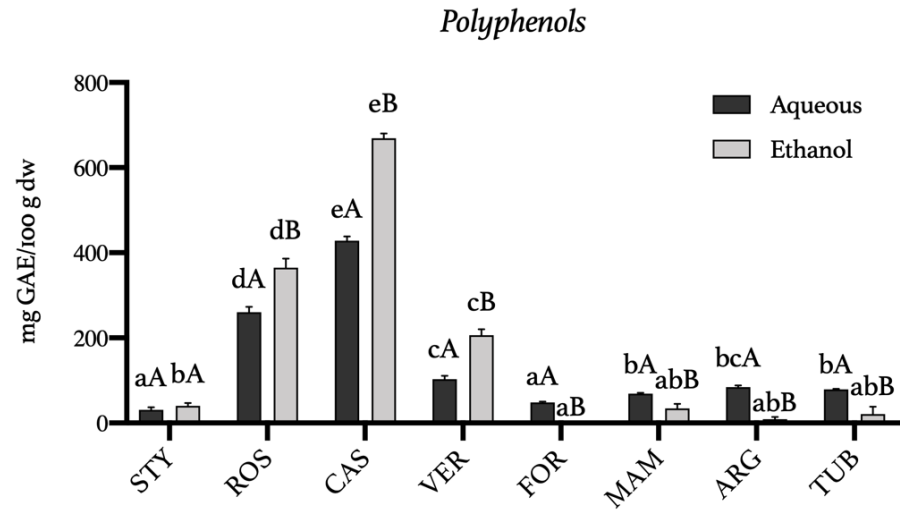


5 min. (x2)



Acquisition by fluorescence microscopy  
- Green filter  
( $\lambda_{em} = 590 \text{ nm}$ ;  $\lambda_{ex} = 546/10 \text{ nm}$ )  
- Magnification: 8.0x

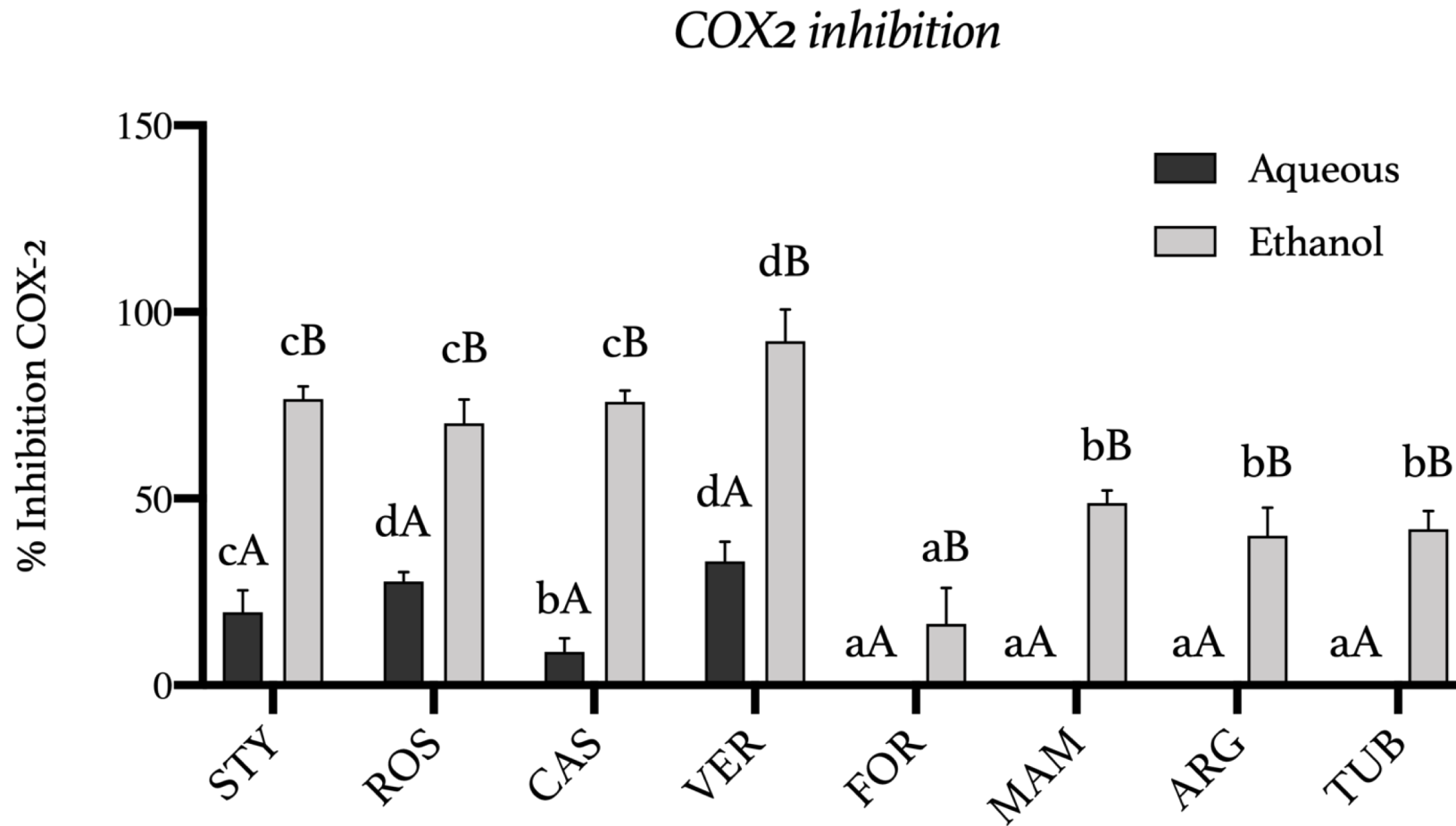
# Results – Anti-oxidant



Values presented as average  $\pm$  SD. nd – not detected. Different lowercase letters correspond to statistical differences ( $p < 0.05$ ) between organisms. Different uppercase letters correspond to statistical differences ( $p < 0.05$ ) between aqueous and ethanolic extracts within species.

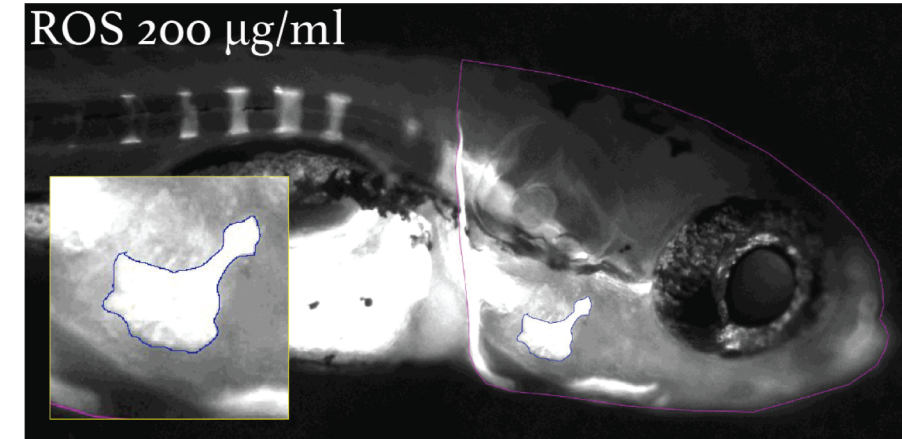
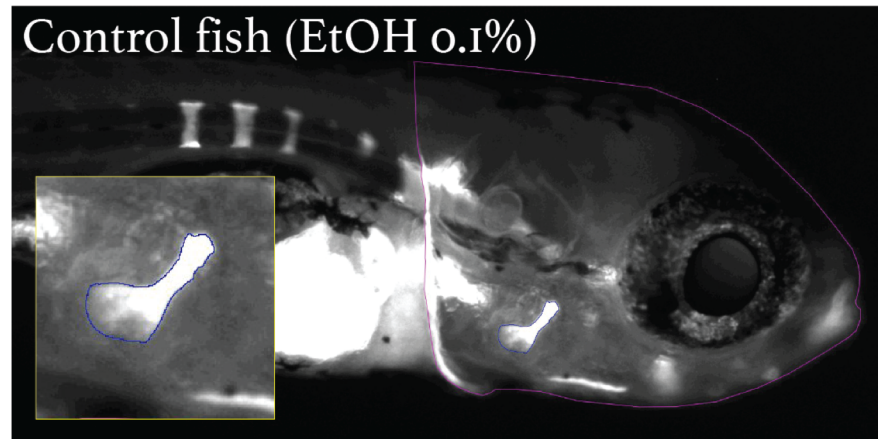


# Results –Anti-inflammatory



Values presented as average  $\pm$  SD. nd – not detected. Different lowercase letters correspond to statistical differences ( $p < 0.05$ ) between organisms. Different uppercase letters correspond to statistical differences ( $p < 0.05$ ) between aqueous and ethanolic extracts within species.

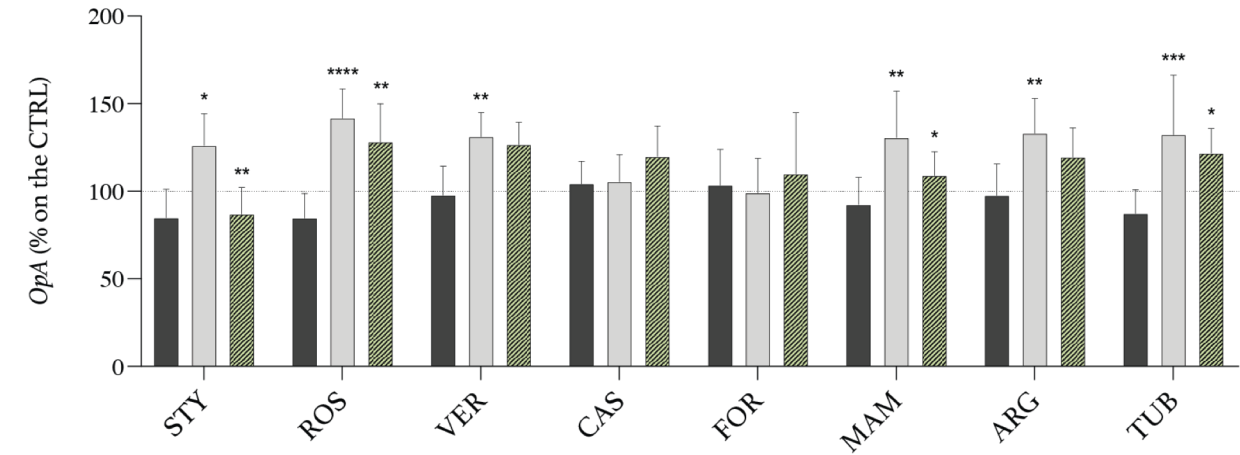
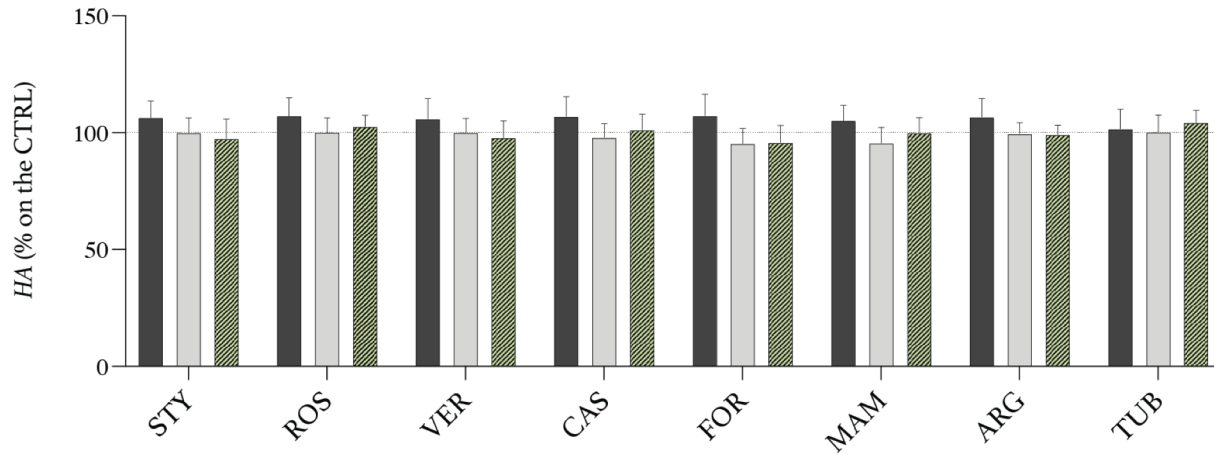
# Results – Osteogenic activity



- Aquous extract
- Ethanol extract
- Ethyl acetate extract

Head Area (HA)

Operculum area



Statistical differences among the means are tested through One-way ANOVA. P values: < 0.0332 (\*), < 0.0021 (\*\*), < 0.0002 (\*\*\*), < 0.0001 (\*\*\*\*).

## Discussion and Conclusions

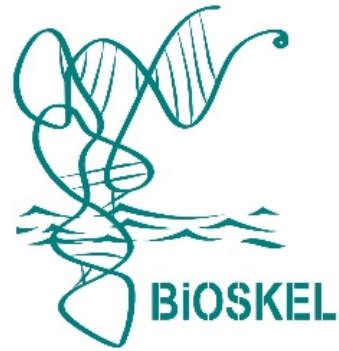
- Ethanolic extracts from all the ascidians (STY, ROS, CAS, VER\*) showed the higher anti-inflammatory activity by strongly inhibiting the cyclooxygenase (COX2) *in vitro*.
- Highest anti-oxidant activity was reported for 3 ethanolic extracts from ascidians (ROS, CAS, VER). However, the antioxidant activity reported may not be related with total polyphenolic content ( $R^2 = 0.29$  for DPPH assay), indicating that other classes of compounds may be responsible for the activity reported.
- Ethanolic extracts of two species of ascidians (ROS and VER) and two species of sea cucumbers (ARG and TUB) induced the highest osteogenic activity.
- Osteogenic and anti-inflammatory activity were significantly correlated ( $R^2 = 0.48$ ).
- Further work is needed to chemically characterise the promising extracts. A possible strategy would be to conduct a bioassay-guided fractionation of the extracts coupled with the application of analytical techniques such as LC-MS, MS<sup>n</sup> and NMR to elucidate the chemical complexity of the fractions and identify the compounds responsible for the reported effects.

\* for species ID refer to the full paper.

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