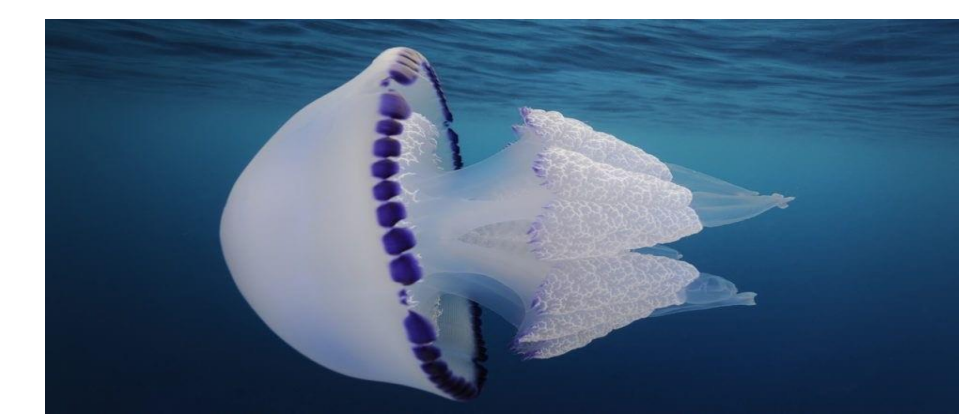


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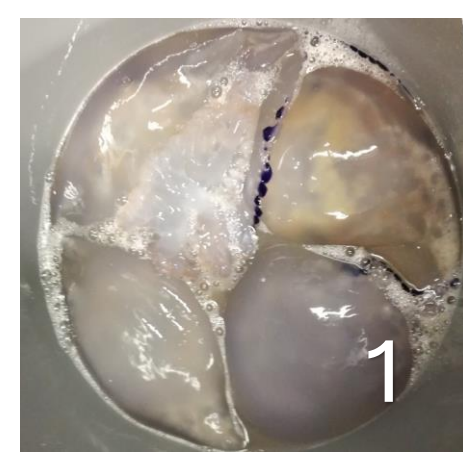


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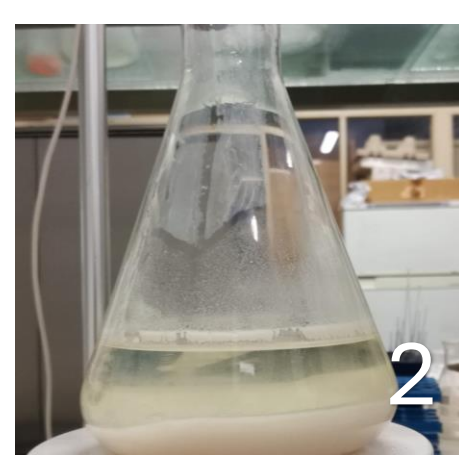


**Introduction:** in recent years, jellyfish has been considered to possess good potential for treating obesity, hypertension, asthma. Previous studies have identified proteins, amino acids, vitamins, and inorganic elements in jellyfish extracts, with a particular focus on macromolecular fractions, especially collagen-like proteins and immunomodulatory polysaccharides (JSPs) as novel functional excipients [1]. The aim of the present work is to compare two different extraction techniques of glycosaminoglycan (GAG)-like polysaccharides from *Rhizostoma pulmo*: conventional hot-water extraction (HWE) and microwave-assisted extraction (MAE).

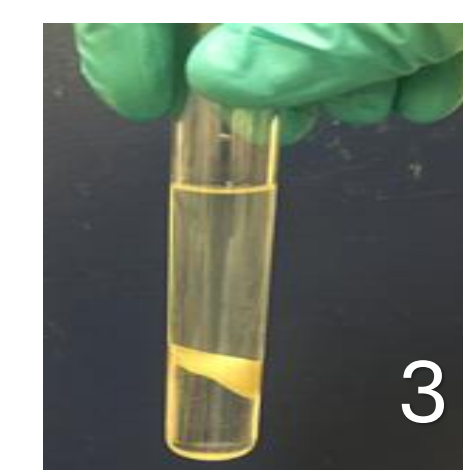
## Hot-water extraction (HWE)



- ✓ Washing
- ✓ Grinding
- ✓ Heating
- ✓ Centrifugation
- ✓ Concentration



- ✓ EtOH precipitation
- ✓ Filtration
- ✓ Drying



- ✓ Removal of proteins Sevag reagent
- ✓ Centrifuged

POST-SEVAG



- ✓ Ion exchange chromatography
- H<sub>2</sub>O elution Not charged P
- NaCl 0.3 M elution Negative P

% extracted polysaccharide: 0.023% w/w

**Abbreviations:**

Post-Sevag Polysaccharide fraction: **Post-sevag**  
Not charged polysaccharide: **Not charged P**  
Negative charged polysaccharide: **Negative P**

## Microwave-assisted extraction (MAE):

- Jellyfishes were removed from tentacles, washed with deionized water and weighted

**Dehydration method**

- Freeze-Thawing cycles (FT)
- Keep in Ethanol 96% 4°C for three days (ETOH)

**Microwave-assisted extraction (MAE)**

- 140°C for 10 minutes (A)
- 120°C for 30 minutes (B)

- Add to supernatant ethanol 96% in ratio 1:4 at 4°C overnight to precipitate protein content

• Sevag method (chloroform:n-butanol=4:1) to remove protein residue

Post-sevag

• Anion exchange chromatography column eluted with:  
1. Ultra pure water  
2. NaCl 0.3 M

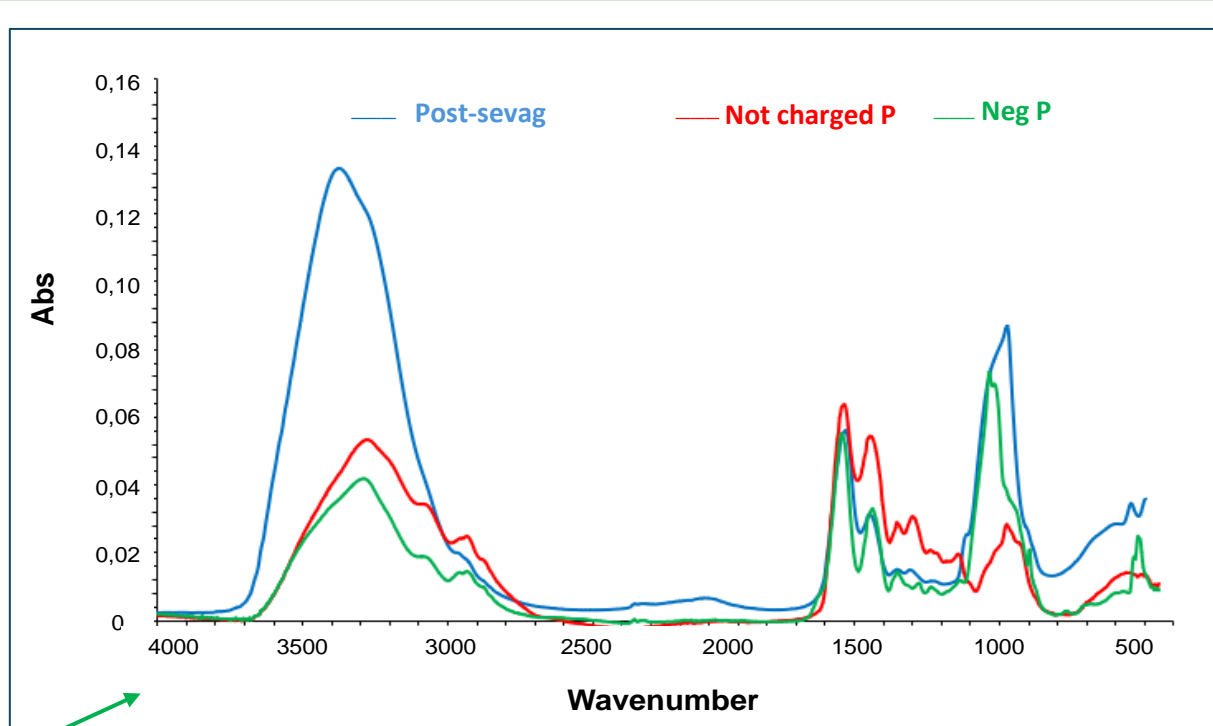
- 1. Not charged P
- 2. Negative P



Single fraction with intermediate chemical-physical properties:  
**Single fraction JSP**

## Physical-chemical characterization

HWE	Post-Sevag	Not charged P	Negative P
<b>Mw (kDa) Debye plot</b>	-	121 ± 6.33	590 ± 13.5
<b>Proteins (% wt) BCA assay</b>	18.42 ± 0.71	25.13 ± 0.78	17.22 ± 0.42
<b>Sulphates (% wt) BaCl<sub>2</sub>-Gelation</b>	24.20 ± 0.11	3.99 ± 0.22	25.92 ± 0.02
<b>ATR-FTIR</b>	1100-1040 cm <sup>-1</sup> polysaccharides, 1240 cm <sup>-1</sup> sulphate group, 1630-1540 cm <sup>-1</sup> proteins/aminoacid/ glucuronic acid		



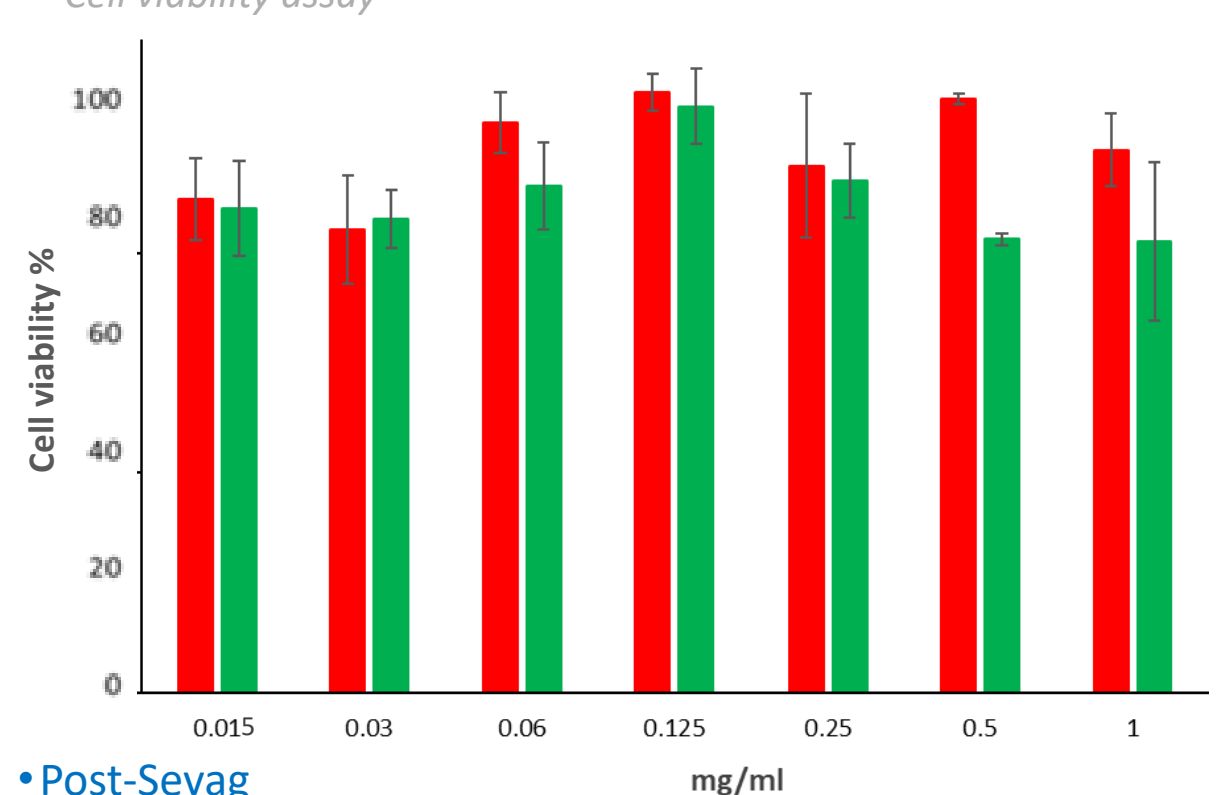
The data obtained from physical and chemical characterization confirm the quality of the extracted JSPs. The combination of FT and B MAE condition seems to be the best alternative to extract JSPs [1]

MAE (conditions B FT)	Post-Sevag	Not charged P	Negative P
<b>Mw (kDa) Debye plot</b>	-	102 ± 11.2	348 ± 17.9
<b>Proteins (% wt) BCA assay</b>	34.4 ± 2.7	24.6 ± 3.6	1.2 ± 0.4
<b>Sulphates (% wt) BaCl<sub>2</sub> - Gelation</b>	11.6 ± 0.4	4.4 ± 1.9	8.3 ± 0.4

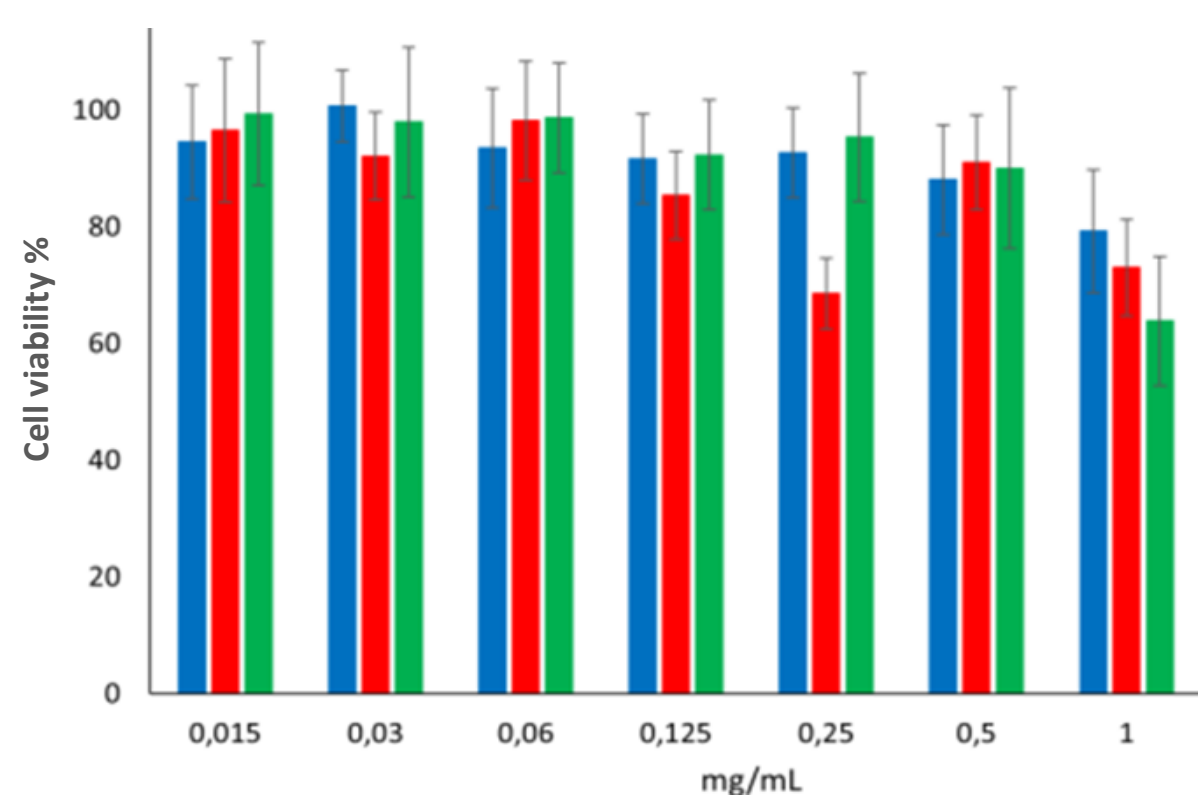
## Biological evaluation

✓ Good cell viability after 24h

Cell viability assay



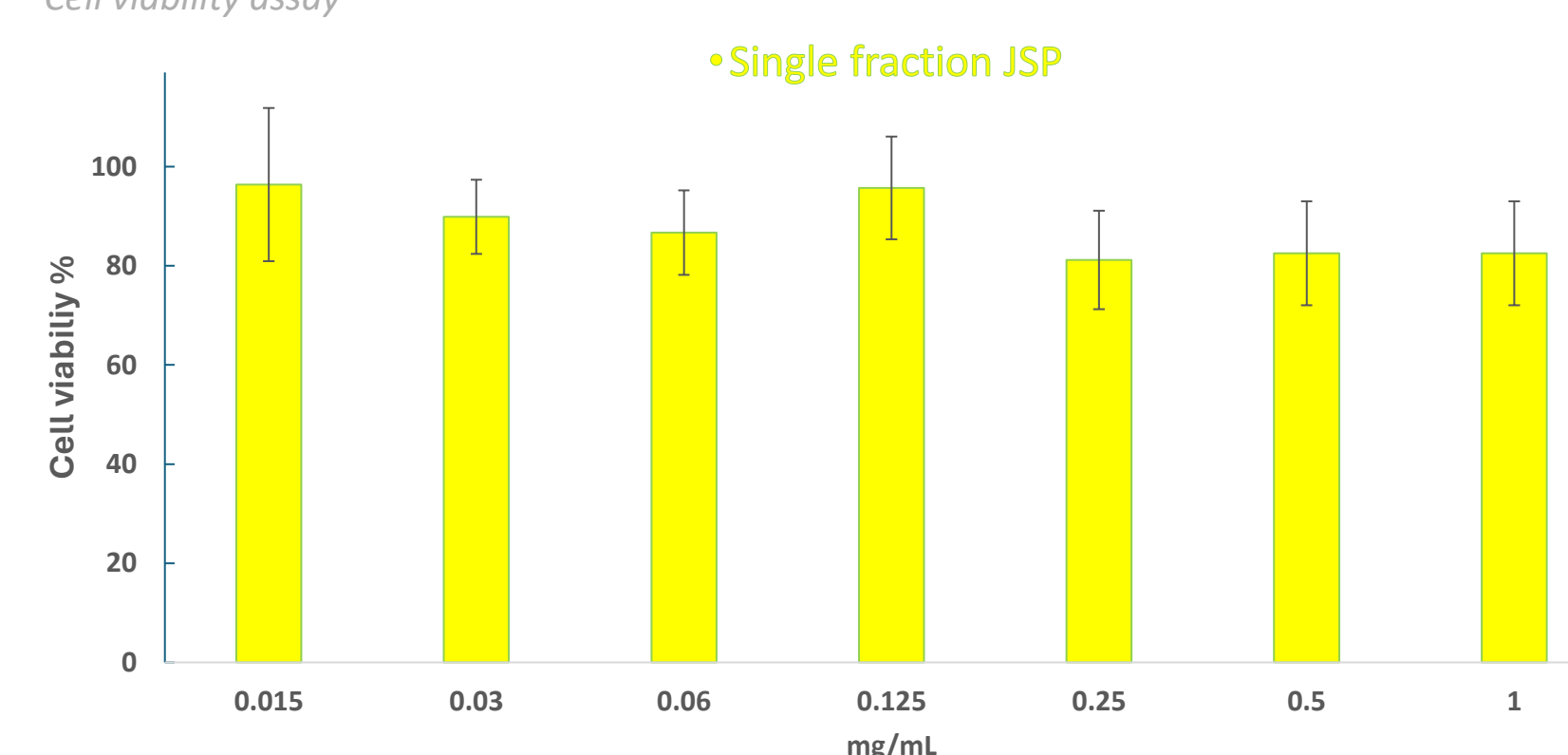
• Post-Sevag  
• Not charged P  
• Negative P



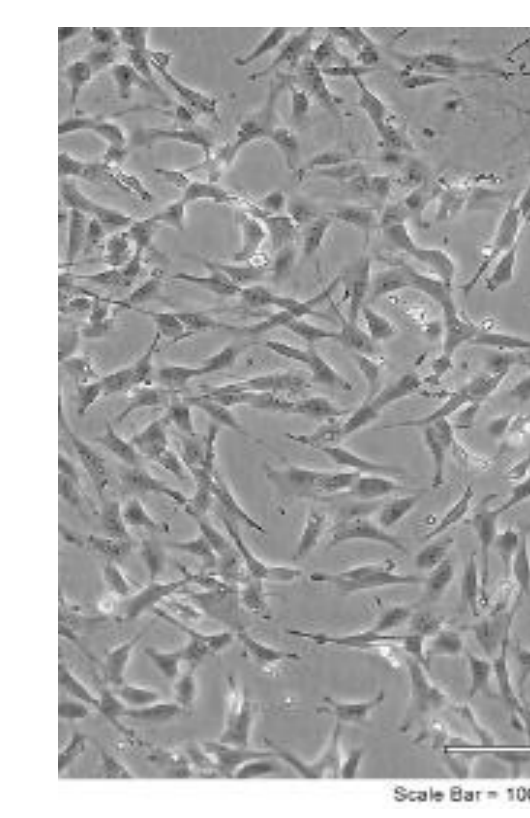
BALB/3T3 Clone A31 Cells-cytotoxicity

✓ Good cell viability after 24h

Cell viability assay



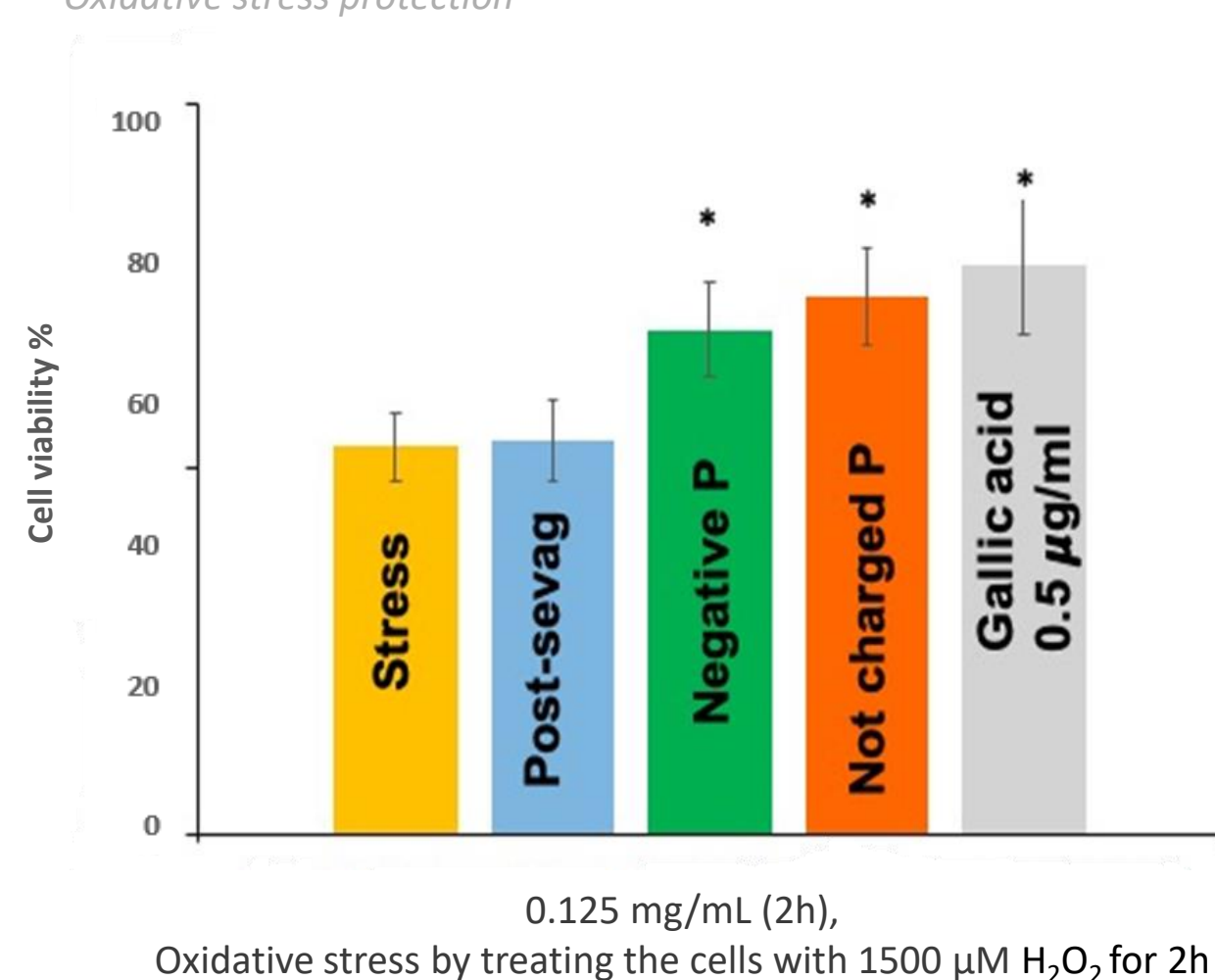
BALB/3T3 Clone A31 Cells-cytotoxicity



BALB/3T3 Clone A31 cells

✓ Protection from oxidative stress

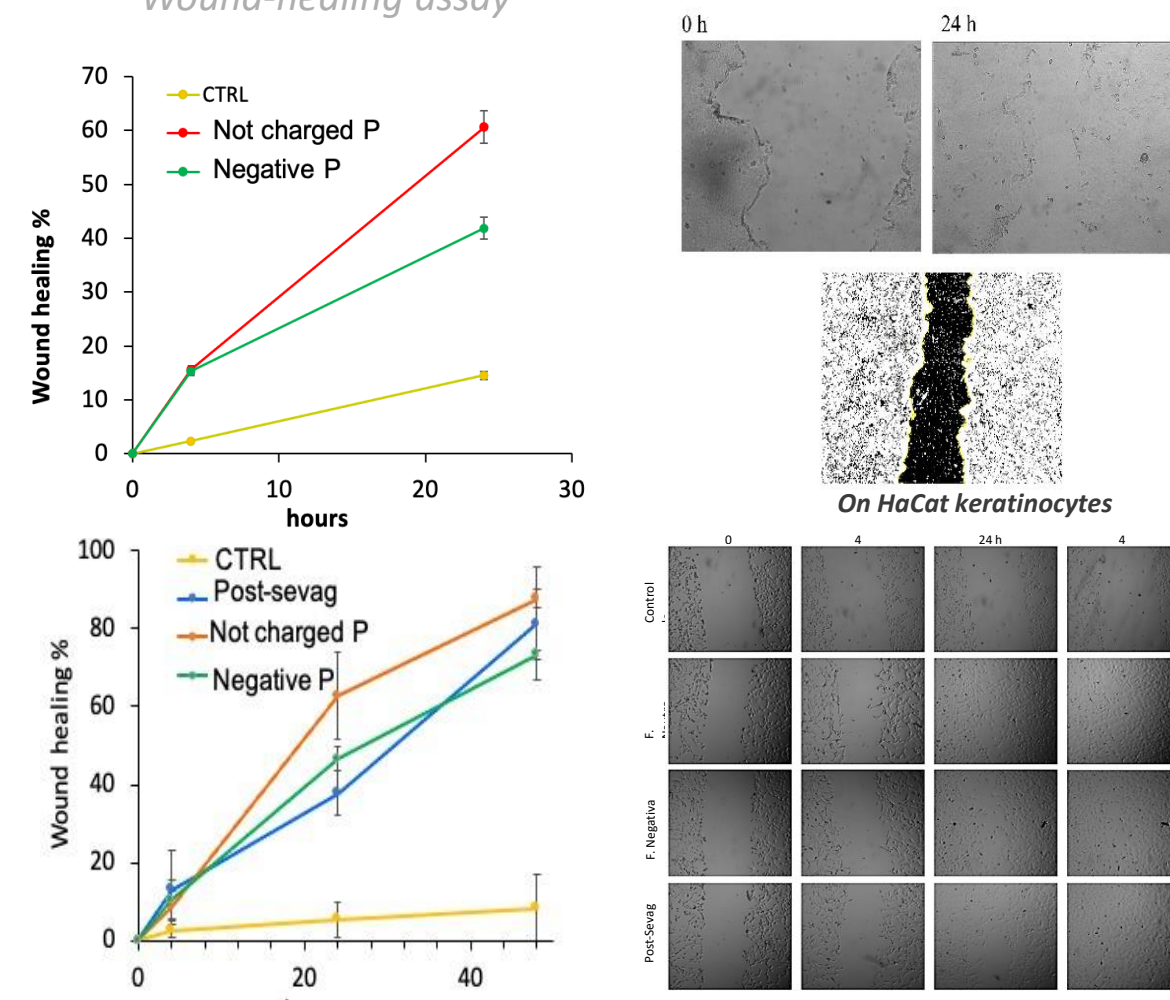
Oxidative stress protection



0.125 mg/mL (2h), Oxidative stress by treating the cells with 1500 µM H<sub>2</sub>O<sub>2</sub> for 2h

✓ Good wound-healing properties

Wound-healing assay

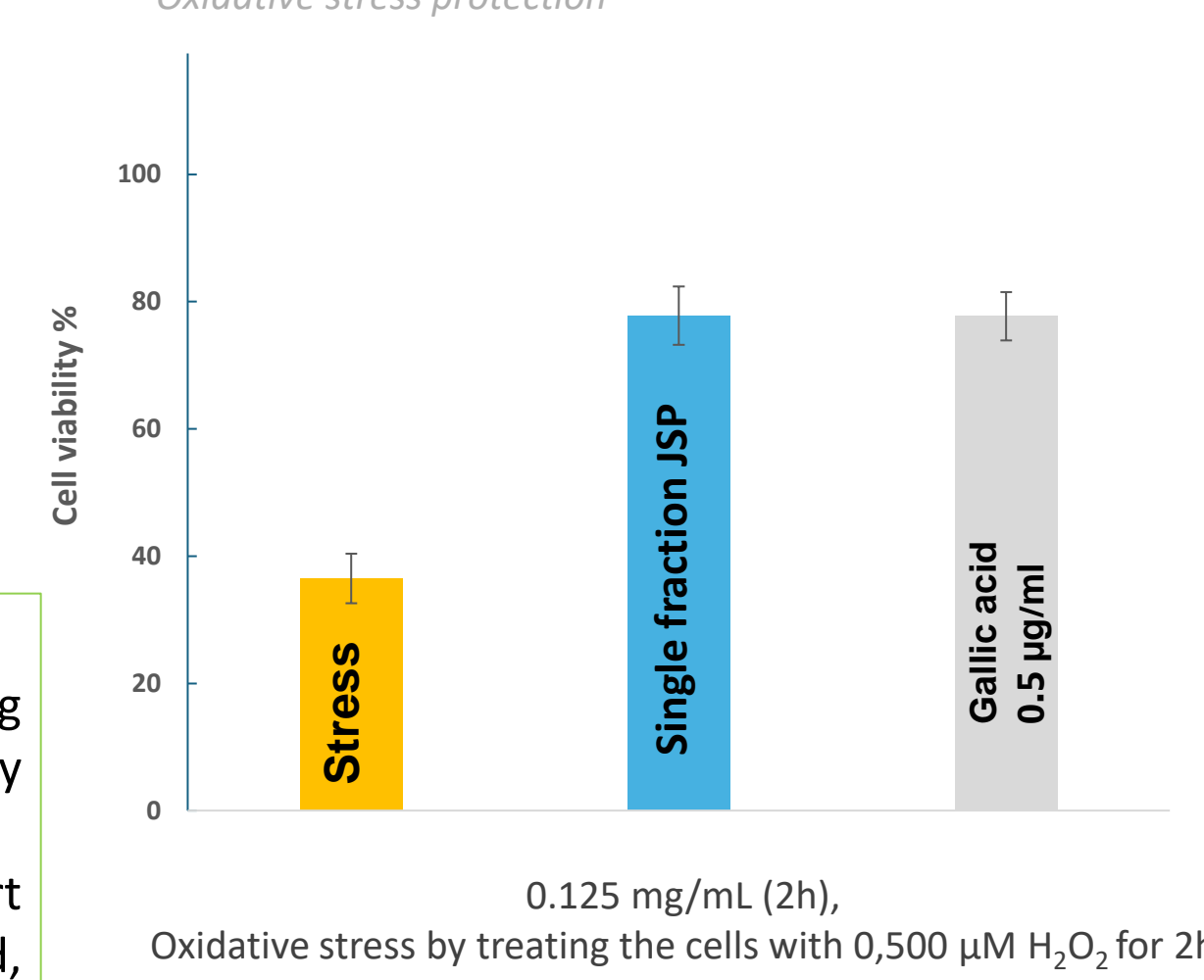


The extracted material showed excellent biocompatibility and an effective in-vitro wound healing activity of more than 75% after 48 hours

The biological properties of the extracted polysaccharides are preserved, providing protection against oxidative stress and enhancing the viability of fibroblast cell lines treated with H<sub>2</sub>O<sub>2</sub>

✓ Protection from oxidative stress

Oxidative stress protection



0.125 mg/mL (2h), Oxidative stress by treating the cells with 0,500 µM H<sub>2</sub>O<sub>2</sub> for 2h

**Future prospective:** Further investigations will involve wound healing assays using the **Single fraction JSP** obtained by MAE. Additionally, the mechanism by which JSPs exert their antioxidant activity will be examined, ensuring their efficacy in biomedical applications

**Conclusions:** Microwave-assisted extraction (MAE) emerges as a more environmentally friendly alternative, reducing extraction times and energy consumption while maintaining the chemical-physical and biological properties of the JSPs. This study highlights the potential of *Rhizostoma pulmo* as a valuable source of marine collagen and GAG-like polysaccharides, proposing MAE as the most sustainable extraction technique. The isolated GAG-like polysaccharides show promising potential as functional excipients, particularly for skin and wound healing applications.

**Reference:** 1 In Vitro Anti-Inflammatory Activity and Structural Characteristics of Polysaccharides Extracted from *Lobonema smithii* Jellyfish by Thitikan Summat et al., *Mar. Drugs* 2023, 21(11), 559; <https://doi.org/10.3390/md21110559>

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