P22

Green synthesis of gold nanoparticles led by algae *Cystoseira tamariscifolia* and evaluation of their biological activity

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In recent years, the development of efficient green chemistry methods for synthesis of metal nanoparticles has emerged as an eco-friendly alternative for the production of well-characterized nanoparticles [1]. Gold nanoparticles are presently under intensive study due to their attractive physicochemical and biological properties as well as their potential applications in the development of new technologies for nanomedicine, both in theraphy and diagnosis [2,3]. Seaweed phytochemicals, including hydroxyl, carboxyl, and amino functional groups, can serve both as effective metal-reducing agents and as capping agents to provide a robust coating on metal nanoparticles in a single step [4].

In this study, we tested if *Cystoseira tamariscifolia (CT)* is a potential agent involved in the reducing and stabilizing processes for the green synthesis of gold nanoparticles. An aqueous extract of this macroalga was prepared and used to produce spherical, stable, polycrystalline nanoparticles with a mean diameter of 7.6 ± 2.2 nm for Au@CT (gold nanoparticles produced in CT extracts), as demonstrated by UV–vis spectroscopy, TEM, HRTEM, STEM and zeta potential measurements. Moreover, the biomolecules present in the extract and nanoparticles were characterized by Fourier Transformed Infrared Spectroscopy (FTIR). *In vitro* antioxidant activity assays also showed that CT extract has a high reducing power, phenolic content and DPPH scavenging activity.

Effects of Au@CT and CT extract alone on cellular metabolism were evaluated by tetrazolium-based colorimetric cellular assay (MTT), and on cell membrane integrity by lactate dehydrogenase activity (LDH) assay. Wound-healing assay revealed impacts on cell proliferation and migration capacity. These assays were performed to investigate whether Au@CT and CT extract alone affect cell viability in mouse (L929 cell line) and human (BJ5-ta cell line) fibroblast cells as *in vitro* models. Lower concentrations of the alga extract and derived nanoparticles did not cause any cytotoxicity. This, together with the wound-healing assay, indicates a potential positive role in cell regeneration.

The zebrafish embryotoxicity (ZET) assay, recomended by OECD to evaluate acute toxicity, was performed to obtain a correlation between *in vitro* and *in vivo* toxicity. These embryos are translucent, allowing direct, real time observation and the evaluation of whole organism responses, from mortality to more specific parameters such as neurotoxicity. The results show that toxicity is evident only at very high concentrations

These results reveal that green synthesis in CT extracts of non-toxic, bioactive nanoparticles have very desirable features with potential applications in biomedicine.

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References:

1. Choudhary R.; Madhuri R. Green Metal Nanoparticles; Scrivener Publishing LLC: **2018**; pp 629-665.
2. Qin L.; Zeng G.; Lai C.; Huang D.; Xu P.; Zhang C.; Cheng M.; Liu X.; Liu S.; Li B.; Yi H. *Coord. Chem. Rev.* **2018**, *359*, 1-31
3. Prado-López S.; González-Ballesteros N.; Rodríguez-Argüelles M. C. Biomaterial in clinical practice. Advances in clinical research and medical devices; Springer: Switzerland, **2018**; pp 407-428.
4. Khan A. U.; Khan M.; Malik N.; Cho M. H.; Khan M. M. *Bioprocess and Biosystems Engineering* **2019**, *42*, 1-15.