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Synergistic Effect of Bioactive Green Macroalgae-Medicinal Plant Hydroalcoholic Mixtures Intended for Skin Repair and Protection

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Abstract: The aim of this study was to investigate the chemical and biological properties of novel green macroalgae-medicinal plants hydroalcoholic mixtures, useful to develop innovative pharmaceutical products for skin repair and protection. The bioactive 20% (w/v) extracts in 70% (v/v) ethanol were prepared by green ultrasound-assisted extraction (26 kHz, 200 W) of mixtures of the green algae *Ulva rigida* (syn. *Ulva lactuca*), collected from the Black Sea, with *Taraxacum officinalis* L. (dandelion) or *Calendula officinalis* L. (marigold) flowers, respectively, at room temperature, for 15 min. The extracts were chemically characterized by total phenolic, carotenoid and chlorophyll content, and their antioxidant activity was determined by photochemiluminescence method, using Trolox as standard. The biological properties of the mixtures were evaluated in terms of *in vitro* cytotoxicity by MTT assay, intracellular reactive oxygen species (ROS) production by flow cytometry, anti-inflammatory activity by ELISA quantification of specific cytokines, regenerative potential by scratch assay and photoprotective effect in an UVB-irradiated experimental model in NCTC fibroblasts and HaCaT keratinocytes. The *Ulva*-plant mixtures exhibited higher antioxidant activity and degree of cytocompatibility (0.1-2 mg/mL on NCTC and 0.1-1 mg/mL on HaCaT), compared to *Ulva* extract (0.1-1 mg/mL on NCTC and 0.1-0.5 mg/mL on HaCaT), indicating their capacity to stimulate cell proliferation. The highest decrease of intracellular ROS production was exhibited by the *Ulva*-marigold mixture, at 0.5 and 0.75 mg/mL, in both oxidative-stressed NCTC and HaCaT cultures. Moreover, *Ulva*-marigold and *Ulva*-dandelion mixtures showed anti-inflammatory activity, reducing TNF-α secretion at a concentration of 1 mg/mL. *Ulva*-marigold mixture presented photoprotective effect in UVB-irradiated cells, and a better capacity to stimulate cell migration into a wounded cell monolayer, than the *Ulva* extract. Overall, the enrichment of *Ulva* extracts with medicinal plants provided bioactive mixtures with synergistic biological properties, which supported their future use in innovative pharmaceutical products.

Keywords: Ulva–Plant Extracts; Antioxidant Activity; Cytocompatibility; Anti-inflammatory Effects; Photoprotection.