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Improving the nutraceutical potential of *Eryngium viviparum* J. Gay through *in vitro* culture elicitation

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Thematic Areas: Food biotechnology.

Abstract: Food degradation by chemical oxidation represents one of the major problems for society. Antioxidants occur naturally in foodstuffs, but can also be added as preservatives to retard or prevent oxidation phenomena, since they are capable of breaking free radical-mediated chain reactions. Artificial antioxidants are the most commonly used compounds by the food industry, but the ingestion of many of these molecules have been linked to possible carcinogenic and toxic effects in humans (1). Therefore, it is necessary to search for new sources of natural antioxidant to help overcome these problems. Moreover, there are species that have interesting bioactive molecules thus they are in extinction and can disappear during time (2). Therefore, *in vitro* culture is a great biotechnological tool used both to conserve threatened plants as to improve the yield of secondary metabolites with antioxidant properties. Thus, this study was carried out to demonstrate the importance of the *in vitro* culture for conservation purposes and to elicit the production of phytochemicals with antioxidant properties in *Eryngium viviparum* J. Gay tissues. Explants of the threatened plant *E. viviparum* were cultured *in vitro* in solidified MS medium and a 3-level factorial design with 2 factors was implemented to studied the effect of cytokinins 6-benzylaminopurine (BAP) and kinetin (KIN) at 0, 1 and 2 mg L^{-1} on the elicitation of antioxidants. Hydroethanolic extracts (ethanol:water 80:20, *v/v*) were prepared from aerial parts and roots of *in vitro* culture by solid-liquid extraction (3). Two cell-based antioxidant activity assays were performed to assess the lipid peroxidation inhibition capacity, through the thiobarbituric acid reactive substances (TBARS) formation inhibition capacity using brain cell homogenates, and the anti-haemolytic capacity, through the oxidative haemolysis inhibition assay (OxHLIA) using sheep erythrocytes (3). The synthetic antioxidant trolox was used as positive control. The antioxidant activities were then correlated with the content of phenolic compounds, which were determined by HPLC-DAD-ESI/MS (4). The root extracts had higher antioxidant activity than aerial parts in both assays and both cytokinins were correlated with the increase of their antioxidant potential. The content of phenolic compounds was also higher in roots and strongly correlated with the recorded antioxidant activity. Regarding the aerial parts, only KIN increased the concentration of antioxidant compounds in the hydroethanolic extracts, but this activity was lower. Therefore, the best results were achieved in root extracts, which had similar antioxidant activity as trolox and the elicitation increased their phenolic compounds content by two times in comparison to the control sample without cytokinins. This study highlighted the interest of *in vitro* culture to obtain *E. viviparum* roots rich in phenolic compounds with high antioxidant activity. Hence, the extracts could be used in the food and nutraceutical industries for several applications as natural antioxidants.

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