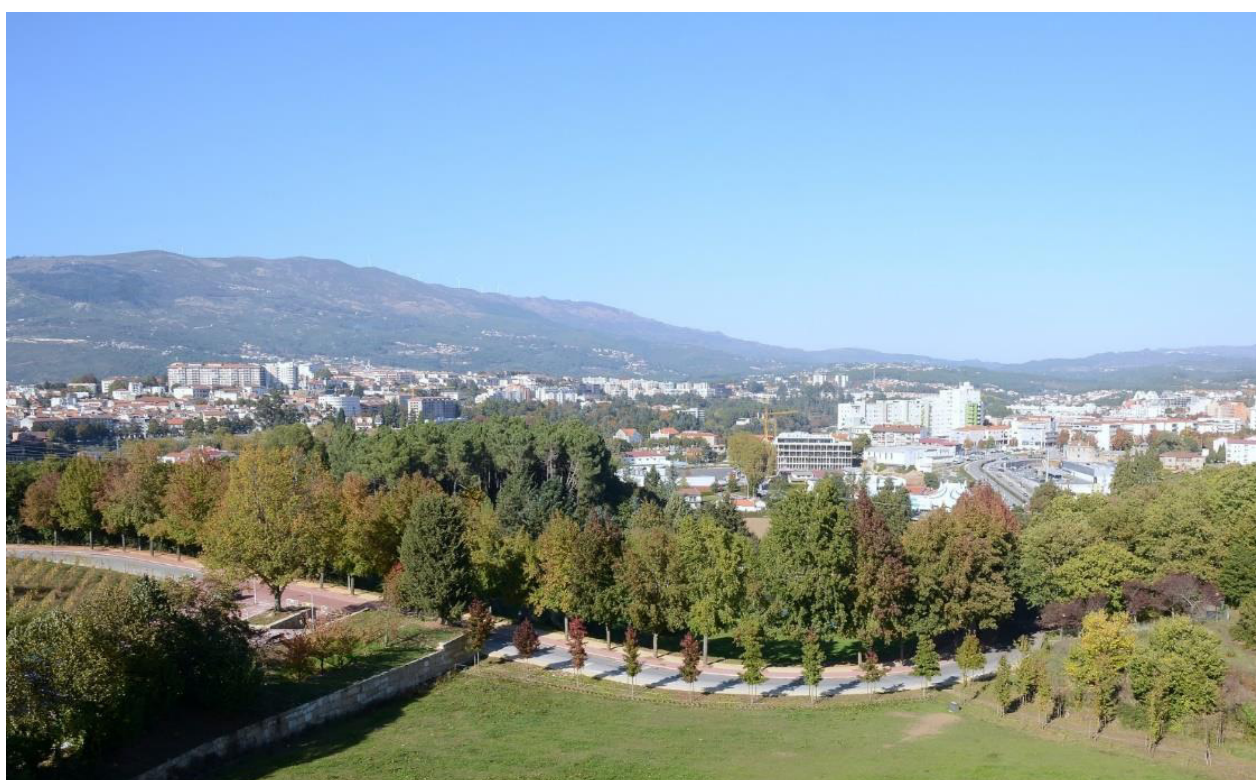





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POMEGRANATE LEAF EXTRACTS CHARACTERIZATION AND THEIR POTENTIAL USE AS A FUNCTIONAL INGREDIENT

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The pomegranate tree (*Punica granatum* L.) is indigenous to the Mediterranean region and is one of the oldest known plants. Studies have demonstrated their many biological and pharmaceutical benefits, which have been correlated to their bioactive content, namely secondary metabolites such as phenolic compounds [1]. However, 40 to 50% of the whole fruit is discarded, such as peel and leaves, originating valuable biowaste [2]. To support a circular economy and the prospective use of pomegranate biowaste as functional ingredients, the present research sought to ascertain the chemical composition and bioactive characteristics of pomegranate leaves. Maceration (ME), microwave (MAE), and ultrasound-assisted (UAE) extractions were the three extraction procedures chosen. HPLC-DAD-ESI/MS was used to identify and quantify the chemical composition of the various extracts. Two cell-based procedures were used to evaluate the antioxidant potential, TBARS and CAA. The antiproliferative potential was studied through sulforhodamine B colourimetric assays, using several tumour and a non-tumour cell lines. The extract's ability to reduce nitric oxide generation was used to determine its anti-inflammatory effects. Lastly, the microdilution technique was used to assess antimicrobial activity and cell viability assay conducted in two skin cell lines to support their possible use in topical formulation. According to the findings, the highest content of phenolic compounds was obtained in the MAE extract. Gallic acid, epicatechin, and granatin B were the most abundant compounds detected in all three extraction methodologies studied. All three extracts demonstrated lower IC₅₀ values (0.83 - 1.70 µg/mL) than the positive control Trolox (IC₅₀ = 9.1 ± 0.3 mg/mL), being the best results obtained for MAE and ME extracts. All extracts showed the capacity to halt the growth of tumour cell lines (GI₅₀ between 19 and 76 µg/mL), being gastric adenocarcinoma (AGS), the cell line with the highest sensitivity. All three extracts demonstrated broad-spectrum antibacterial inhibition, in which *K. pneumoniae* displayed the highest sensitivity to the extracts (MIC values of 0.6 mg/mL). In the tested skin cell lines, none of the studied extracts had any observable adverse effects, and more than 50% of viability was maintained after exposure to the highest tested concentrations of each extract (400 µg/mL). According to our findings, pomegranate leaves appear to be a source of health-promoting biomolecules. Those results sustain their exploitation and possible use by food and pharmaceutical industries in some biobased products, enhancing the circular economy and zero biowastes.

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