*Original Research Article*

Antioxidant activity of pomegranate peel extracts (lat. *Punica granatum L.*): Effects of extraction solvent and technique on antioxidant activity

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ABSTRACT

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| This study focuses on evaluating the antioxidant activity of pomegranate (lat. *Punica granatum L*.) peel extracts obtained through various extraction methods, including ultrasound-assisted extraction (UAE), Soxhlet extraction, and maceration, using methanol and 96% ethanol as solvents. These techniques were chosen for their differing effects on the preservation of thermosensitive phytochemicals. Antioxidant potential was assessed using the DPPH radical scavenging assay, with absorbance measured at 517 nm using a UV-Vis spectrophotometer. The results demonstrated that the efficiency of antioxidant activity largely depended on the extraction method and solvent used. A lower IC50 value indicates higher antioxidant activity, as it reflects a greater efficiency in neutralizing free radicals at a lower concentration. Extracts obtained by ultrasound-assisted extraction exhibited the highest radical scavenging capacity with an IC50 value of 19.049 μg/mL, while those obtained by Soxhlet extraction with ethanol showed comparatively weaker activity with an IC50 value of 34.210 μg/mL, likely due to the thermal degradation of sensitive bioactive compounds. The maceration method, although mild and solvent-efficient, yielded moderate antioxidant activity, highlighting the balance between extraction intensity and preservation of functional constituents. The study emphasizes the importance of optimizing extraction conditions to maximize the recovery of bioactive compounds from plant materials. Given the phytochemical richness and biological potential of pomegranate peel, the findings support its application as a natural source of antioxidants in the development of dermocosmetic and pharmaceutical formulations aimed at combating oxidative stress and disorders related to hyperpigmentation and skin aging. |

*Keywords: Pomegranate peel; Extraction; UAE; Soxhlet extraction; Maceration; Phenolic compounds; Antioxidant activity; DPPH.*

1. INTRODUCTION

Pomegranate (lat. *Punica granatum L.*) is a fruit-bearing shrub that belongs to the Punicaceae family. The pomegranate is rich in phytochemicals that have antioxidant, anti-inflammatory, anti-diabetic, and cardiovascular effects, as well as antidyslipidemic, antibacterial, antiviral, and anticancer properties. The phytochemicals include organic acids such as citric acid, isocitric acid, and dimethyl ester of citric acid, phenolic acids (i.e., chlorogenic acid, gallic acid, gallic acid derivatives, ferulic acid, p-coumaric acid hexosides, phloroglucinol acid, cinnamoylrhamnoside, and homovanillic acid), which are attributed with antioxidant effects, neutralizing free radicals, positively influencing the cardiovascular system, and reducing LDL. (Bhise et al., 2025; Dalvi et al., 2017) It is important to emphasize that chlorogenic acid has a positive effect on individuals with diabetes, as it reduces glucose absorption, improving its regulation. A significant portion belongs to flavonoids such as catechin, hyperoside, kaempferol, luteolin, and luteolin derivatives, which have similar beneficial effects as the previously mentioned components. The main secondary metabolites are tannins, including ellagitannins and gallotannins, which have astringent, anti-inflammatory, and antioxidant effects. (Benedetti et al., 2023) Skin pigmentation depends on the amount of melanin synthesized in melanocytes. Hyperpigmentation represents an increased synthesis of melanin due to higher activity of the enzyme tyrosinase in melanocytes of the epidermal layer of the skin. The biosynthesis of melanin is catalyzed by the enzyme tyrosinase through the oxidation of L-tyrosine to L-dopa and subsequently L-dopa to dopachrome, followed by the formation of melanin. (Maeda et al., 1997) *Punica granatum L.*, due to its content of polyphenols and tannins, leads to the inhibition of tyrosinase catalytic activity and a reduction in melanin synthesis, the brown skin pigment, thus decreasing skin pigmentation. Among polyphenols, punicalagin plays the most important role, a polyphenol from the group of ellagic acid, which is known for its antioxidant properties. (Rana et al., 2013) Antioxidant activity of phenolic compounds found in pomegranate peel is reflected in their ability to form complexes with metal ions and remove free radicals that lead to oxidative stress. (Dalvi et al., 2017) In the study by Yang et al., ellagic acid demonstrated significant anti-melanogenic effects by inducing autophagy in melanocytes and suppressing UVA-activated α-MSH pathways through Nrf2 activation in keratinocytes. (Yang et al., 2021) This article will present the results of the antioxidant activity of pomegranate peel extracts using the DPPH Assay, revealing the significance of a suitable selection of extraction method and extraction solvent.

1. material and methods

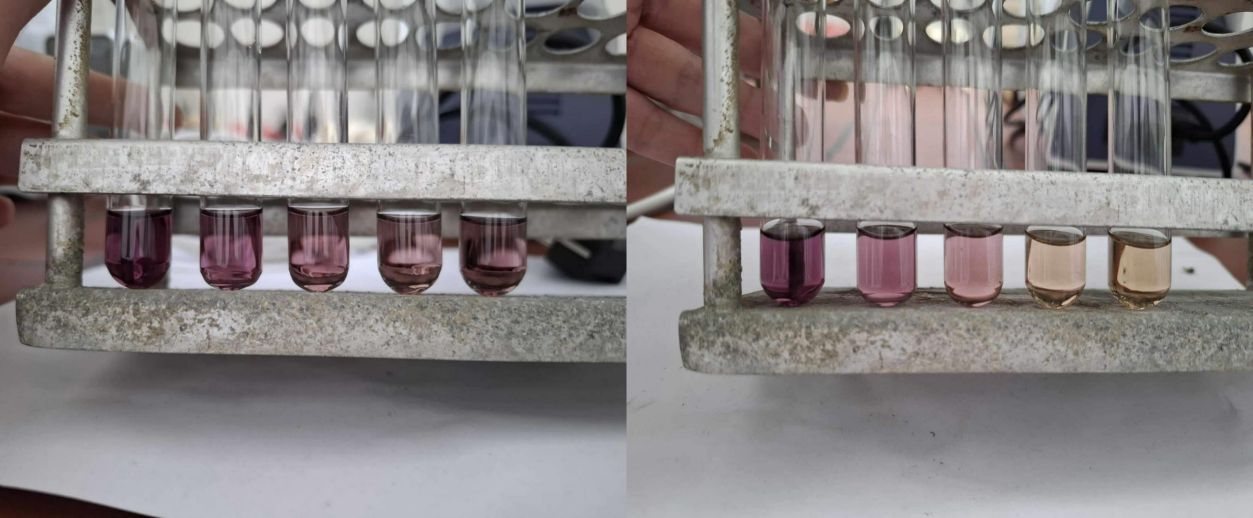
Dried pomegranate peel (lat. *Punica granatum L.*) was used as the plant material for extraction.The peel was washed in clean water to remove any impurities, dust, or potential pesticide residues, and then gently wiped with a clean cloth, cut into smaller pieces, and left to air dry completely, preventing excess moisture that could interfere with further processing. The drying process was carried out in a well-ventilated area at room temperature for 3 to 4 days to preserve sensitive bioactive compounds. The dried pomegranate peel was then finely ground in an electric mill and stored at room temperature. (Ahmetović et al., 2025)

* 1. **Preparation of Extracts**

Pomegranate peel extracts were prepared by ultrasound-assisted extraction (UAE), Soxhlet extraction, and maceration, using methanol and 96% ethanol as extraction solvents. Both solvents were used for UAE and Soxhlet extraction to obtain both ethanolic and methanolic extracts, while for maceration, only 96% ethanol was used as an extraction solvent. The solvent-to-sample ratio used for UAE was 20:1 (vol/vol), and 10:1 (vol/vol) for the Soxhlet extraction method and maceration. For the UAE, 2.5 grams of crushed peel material were transferred into a flat-bottom flask and poured with 50 mL of solvent (methanol and 96% ethanol). UAE was performed in a Bandelin ultrasonic bath for 35 minutes at room temperature. Soxhlet extraction was performed using 20 grams of finely ground dried pomegranate peel that was placed into the filter paper thimble and inserted into the extraction chamber of the Soxhlet apparatus. 200 mL of solvent (methanol and 96% ethanol) was added to the apparatus through the funnel above the condenser. The extraction proceeds for 6 hours, maintaining a temperature between 60 and 80 °C. For maceration, 1.5 grams of crushed pomegranate peel were soaked in 15 mL of solvent called menstruum (96% ethanol) in a laboratory beaker for 3 days at room temperature with occasional stirring. (Ahmetović et al., 2025) Due to the evaporation of methanol at room temperature, only 96% ethanol was used as the extraction solvent for maceration. After extraction, solvents were removed to obtain the dry pomegranate peel extracts used in further analysis.

* 1. **DPPH Radical Scavenging Assay**

The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method was used to determine the antioxidant activity of pomegranate peel extracts. It is based on the neutralization of the stable free radical DPPH, which in the presence of antioxidants loses its characteristic purple color. (Blois, 1958; Brand-Williams et al., 1995) A Stock solution (1 mg/mL) of the extract was prepared in methanol and used to obtain a series of dilutions, as shown in Fig. 1. Then, 0.5 mL of a 0.5 mM DPPH solution prepared in methanol was added, and the samples were incubated for 30 minutes in a dark room at room temperature. Absorbance was measured at 517 nm with methanol as a blank. The control solution was prepared by mixing 1 mL of 0.5 mM DPPH solution and 4 mL of methanol.



**Figure 1.** A series of dilutions of the extract before and after incubation, from lowest to highest concentration. Color changes indicate the neutralization of the stable free radical DPPH, from purple to yellow.

The radical scavenging effect (%) or DPPH radical inhibition percentage was calculated according to the equation:

[(Ac – As) / Ac] x 100 [%]

where As is the absorbance of the solution containing the sample at 517 nm, and Ac is the absorbance of the control. (Kolarević et al., 2023) The results of the DPPH assay are expressed as IC50 (µg/mL), defined as the concentration of extract required to scavenge 50% of free radicals. (Mensor et al., 2001; Cilović-Kozarević et al., 2022)

1. results and discussion

The antioxidant capacity of the pomegranate peel extracts was evaluated using the DPPH method, with results expressed as IC50 value (µg/mL), as shown in Fig. 2. For the analysis of each extract, a Stock solution (1 mg/mL) was prepared. The Stock solution was used to obtain samples with a range of concentrations from 5 to 25 µg/mL, with the exception that the 96% ethanol extract obtained via Soxhlet extraction was used to prepare samples with a range of concentrations from 20 to 50 μg/mL. Absorbance value of each sample and control solution was measured at 517 nm with methanol as a blank, and used for the calculation of DPPH radical inhibition percentage (%). Results were used to obtain a graph and expressed as an IC50 value (μg/mL). A lower IC50 value indicates higher antioxidant activity, as it reflects a greater efficiency in neutralizing free radicals at a lower concentration. The highest antioxidant capacity was measured for the ethanolic extract obtained by ultrasound-assisted extraction (UAE) with an IC50 value of 19.049 μg/mL. The lowest antioxidant capacity, reflected in the highest IC50 value, was measured for the ethanolic extract obtained with the Soxhlet extraction method, with a value of 34.210 μg/mL. Ethanolic extract obtained with the maceration extraction method exhibited intermediate antioxidant activity with an IC50 value of 26.677 μg/mL. Methanolic extracts exhibited only slightly higher IC50 values compared to the ethanolic extract obtained via UAE, indicating marginally lower antioxidant activity. Measured IC50 value for methanolic extract obtained with UAE was 19.751 μg/mL, while the IC50 value for methanolic extract obtained with Soxhlet extraction method was slightly higher, 21.395 μg/mL. Extracts obtained with the ultrasound-assisted extraction method show higher antioxidant capacity in comparison to the Soxhlet extraction method and the traditional maceration extraction method. A potential explanation for this lies in the fact that the UAE is carried out at or near room temperature, which helps to preserve thermolabile phenolic compounds, as key contributors to antioxidant activity. (Chemat et al., 2011; Huang et al., 2025) Extracts obtained using ultrasound-assisted extraction showed only slight differences in IC50 values depending on the solvent used, suggesting that the method works well regardless of solvent choice. Ethanol performed slightly better, likely due to its semipolar nature, which helps extract a wider variety of antioxidant compounds. (El Mannoubi, 2023; Mohammed et al., 2022) Additionally, ethanol stands out as a safer, non-toxic alternative to methanol, making it more suitable for use in food and pharmaceutical applications. (Spigno et al., 2007; Lee et al., 2024) In contrast, the Soxhlet extraction method operates at elevated temperatures for extended periods, which can result in thermal degradation of phenolic compounds and, accordingly, lower antioxidant capacity. (Shi et al., 2022) Additionally, the UAE also utilizes ultrasound waves to disrupt the plant matrix and enhance the penetration of solvents, unlike the maceration extraction method, which relies solely on passive diffusion over extended periods. (Dai & Mumper, 2010; Chemat et al., 2011) Results have shown that the IC50 value can significantly depend on the solvent used during extraction. In general, methanol extracts obtained with the UAE and Soxhlet extraction method show higher antioxidant capacity and lower IC50 values in comparison to the 96% ethanol extracts obtained with Soxhlet extraction method and maceration, with the exception that the 96% ethanol extract shows the highest antioxidant capacity and the lowest IC50 value. Studies have demonstrated that the higher polarity of methanol enables more efficient extraction of highly polar phytochemicals, particularly phenolic compounds, resulting in extracts with higher total phenolic content, lower IC50 values, and higher antioxidant activity. Methanol’s ability to form hydrogen bonds with polar molecules allows it to extract a broader range of hydrophilic antioxidants. (Boeing et al., 2014) However, because of its toxicity, methanol is not ideal for food or pharmaceutical use, which is why ethanol is often preferred. Ethanol, as a less polar solvent than methanol, can be more selective to moderately polar compounds, such as flavonoids, tannins, and ellagitannins. (Sultana et al., 2009; Dai & Mumper, 2010; Fawole et al., 2012; Lee et al., 2024) These findings indicate that certain bioactive compounds in pomegranate peel, such as ellagic acid, which exhibit moderate polarity, are more efficiently extracted under conditions that combine ultrasound-assisted extraction (UAE) with high-purity ethanol (96%), thereby enhancing the overall antioxidant capacity of the extract.

**Figure 2.** Results of antioxidant activity obtained by the DPPH method, expressed as IC50 value (μg/mL). A lower IC50 value indicates a higher antioxidant activity, while a higher IC50 value indicates lower antioxidant activity.

1. ConclusioN

In an era when natural sources are increasingly recognized for their role in promoting health and supporting the treatment of various conditions, plants such as pomegranate (Punica granatum *L*.) are rightfully garnering scientific attention. The complex chemical composition of pomegranate peel, especially its richness in potent antioxidant compounds, opens promising opportunities for the development of innovative therapeutic and cosmetic products that combine natural origin with proven efficacy.

This study demonstrated that pomegranate peel extracts exhibit marked antioxidant activity, which is significantly influenced by the choice of extraction method and solvent. The findings emphasize that ultrasound-assisted extraction (UAE), particularly when combined with high-purity ethanol, enables more efficient preservation and recovery of thermolabile polyphenolic compounds such as punicalagin, ellagitannins, flavonoids, and phenolic acids. These compounds are known for their biological effects, including tyrosinase inhibition and suppression of melanin synthesis, making them valuable active ingredients in natural skin care products, especially in formulations targeting hyperpigmentation.

The results clearly show that the extraction technique is not merely a technical step, but a crucial factor in preserving the pharmacological potential of herbal raw materials. Moving forward, future research should aim to identify and quantify the specific bioactive compounds responsible for the observed antioxidant effects and assess their biological activity in vivo. Such studies will be essential in developing safe, stable, and effective pharmaceutical and cosmetic formulations based on pomegranate peel extract.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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