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Optimization of ultrasound-assisted extraction from *Olea europaea* leaves and analysis of their antioxidant and enzyme inhibition activities

Sengul Uysal [a,b,*], Biljana Lončar [c], Aleksandra Cvetanović Kljakić [c], Gokhan Zengin [d]

[a] Erciyes University Halil Bayraktar Health Services Vocational College, Kayseri, Türkiye
[b] Drug Application and Research Center, Erciyes University, 38280, Kayseri, Türkiye
[c] Faculty of Technology Novi Sad, University of Novi Sad, 21000, Novi Sad, Serbia
[d] Biochemistry and Physiology Research Laboratory, Department of Biology, Science Faculty, Selcuk University, Konya, 42130, Türkiye

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ABSTRACT

Olive leaves (*Olea europaea* L.) are rich source of bioactive compounds but are usually declared as bio-waste. Knowledge from traditional medicine, supported by modern research, has shown the high value of this bio-waste, and the great benefits of its use. The first step in exploiting the biological potential of olive leaves is the rationalization of the extraction process, through which extracts with high biological and market value could be obtained. This study aimed to investigate the influence of different extraction parameters, including solvent concentration, time, temperature, and ratio, on total phenolics, total flavonoids, antioxidant and enzyme-inhibiton activities. The optimal extraction conditions were determined using an Artificial Neural Network (ANN) model. The results revealed that the optimal conditions for the extraction of total bioactive compounds and antioxidant activities from olive leaves were obtained using ethanol concentration of 90%, extraction time 15 min, temperature of 45 °C, and plant:ethanol ratio 1:30. Under these conditions, the extract exhibited high levels of total phenolic content (TPC: 89.05 ± 3.9 mg GAE/g), total flavonoid content (TFC: 19.98 ± 0.38 mg RE/g), and potent antioxidant activity (DPPH: 195.12 ± 6.85 mg TE/g, ABTS: 270.73 ± 1.00 mg TE/g, CUPRAC: 66.14 ± 25.27 mg TE/g, FRAP: 233.58 ± 21.33 mg TE/g). Additionally, the extract demonstrated significant inhibitory effects on α-amylase (0.35 ± 0.00 mmol ACE/g) and tyrosinase (49.23 ± 1.22 mg KAE/g) enzymes. Taken together, the presented results may be valuable for the preparation of health-promoting formulations using olive leaves in the pharmaceutical and cosmeceutical industries.

1. Introduction

Olive trees (*Olea europaea* L.) are widely cultivated in Mediterranean countries for the production of olive oil (Simat et al., 2022). Olive oil provide well-know health benefits and several research have investigated the enrichment of olive leaves (Marinaccio, Zengin, Bender, Cichelli, et al., 2024a; Marinaccio, Zengin, Bender, Dogan, et al., 2024b). The processing of olives generates significant amounts of by-products and waste, which are typically characterized by low economic value and challenges related to their disposal. The main bio-waste of olive tree cultivation is leaves (Medfai et al., 2020). However, this part of the plant is widely used in folk medicines of many nations for the treatment of infectious and non-infectious diseases (Tsimidou & Papoti, 2010). Olive leaves are recognized as a rich source of phenolic compounds, often surpassing both olive oil and fruit in their concentration of these bioactive molecules. Among the most prevalent phenolic compounds identified in olive leaves are oleuropein, rutin, verbascoside, tyrosol, hydroxytyrosol, apigenin-7-O-glucoside (Khelouf et al., 2023; Martinez-Navarro et al., 2023). These compounds are associated with a range of significant biological activities, including antioxidant, anti-inflammatory, neuroprotective, antimicrobial (Ghalandari et al., 2018; Kermanshah et al., 2020; Romero-Márquez et al., 2022; Tamasi et al., 2019). Given these properties, olive leaves extract has garnered attention for its potential applications in both cosmetic and pharmaceutical industries as a valuable source of natural bioactive molecules (Barbaro et al., 2014).

Due to the rich phytochemical content, particularly its phenolic compounds, there is a growing need to explore sustainable methods for this waste valorization. Ultrasound-assisted extraction (UAE) is one of the innovative extraction techniques suitable for extracting different

* Corresponding author. Erciyes University Halil Bayraktar Health Services Vocational College, Kayseri, Türkiye.
E-mail address: senguluysal@erciyes.edu.tr (S. Uysal).

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