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Bioactive Potential and Phytochemical Profile of Phoenix dactylifera L. Seeds Extracted Using Hydrophilic and Hydrophobic NADES

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Introduction
Date palm consumption produces large amounts of seed by-products rich in polyphenols, carotenoids, tocopherols and other bioactive components.1 These compounds contribute to structural, signaling and immune functions. Natural Deep Eutectic Solvents (NADES) offer a sustainable alternative to conventional solvents, efficiently extracting these valuable biocompounds through a greener process.2 This study evaluates the physicochemical properties and biological activities of date seed extracts produced using both hydrophilic and hydrophobic NADES.

Materials and Methods
Date seed extracts were obtained after heating-stirring (HS) - NADES extraction with a 1:9 (w/v) solid-to-liquid ratio for 45 minutes at 45 °C. Two hydrophilic (Betaine:Urea, Bet:U (1:2) and Betaine:Glycerol:Glucose, Bet:Gly:Glu (4:20:1)) and two hydrophobic NADES (Octanoic acid: Dodecanoic acid, C8:C12 (3:1) and Octanoic acid: L-Proline, C8:Pro (4:1)) with its respective controls (ethanol–water mixture, 50% (v/v), and hexane), were used in this study to evaluate the bioactive potential and profile of date seed, using SDS-PAGE, Bradford assay, activity of the pepsin, trypsin and chymotrypsin determination and MTT assay with differentiated Caco-2.3,4

Results
SDS-PAGE analysis revealed that all hydrophilic NADES extracts exhibited distinct protein bands, with a predominant band consistently observed between 60 and 67 kDa. Among them, the Bet:U extract displayed the highest number and intensity of protein bands. In contrast, only one hydrophobic solvent, C8:Pro, produced visible protein bands, showing a faint band at approximately 66.2 kDa.
According to the Bradford assay, Bet:Gly:Glu was the hydrophilic extract with the highest quantified protein content (7.6 mg protein/g extract). Among hydrophobic extracts, C8:Pro was the only sample in which total protein content could be quantified (0.3 mg protein/g extract).
Based on its superior total polyphenol content compared with the other NADES extracts, Bet:U was selected for the evaluation of pepsin, trypsin, and chymotrypsin activities, as well as for the MTT assay. This extract enhanced the activity of all three enzymes and preserved Caco 2 cell viability up to 20 µL/mL, demonstrating its potential to confer protection against cellular damage.

Conclusions
Hydrophilic NADES demonstrated a superior capacity for protein extraction compared with hydrophobic solvents, as evidenced by the presence of well-defined protein bands in SDS-PAGE, particularly the predominant 60–67 kDa band common across all hydrophilic formulations. Among these, Bet:U showed the highest capacity of protein extraction. In protein extraction, Bet:Gly:Glu and Bet:U yielding the highest protein content without statistical differences, while only one hydrophobic solvent (C8:Pro) enabled measurable protein recovery.
Bet:U, selected due to its elevated polyphenol concentration, enhanced pepsin, trypsin and chymotrypsin activities and maintained Caco-2 cell viability. These findings highlight Bet:U as a promising extraction medium capable of preserving bioactive compounds activity and conferring protective effects against cellular stressors, including oxidative agents and indomethacin. Overall, NADES—especially hydrophilic formulations—emerge as effective and biologically compatible solvents for recovering functional biomolecules.

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