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Using alternative solvents for the extraction of phenolic compounds: Juglans regia L. leaves as a case study

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PURPOSE OF THE ABSTRACT

Juglans regia L. (walnut) leaves are recognised as a rich source of bioactive molecules, such as phenolic acids and flavonols. The main aim of this work is to study the use of eutectic solvents to extract phenolic compounds and evaluate the bioactive potential of the extracts obtained. A set of solvents frequently described as hydrogen bond donors in deep eutectic solvents were used for a preliminary screening: ethanol, glycerol and a series of diols (1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 1,3-butanediol, 1,2-pentanediol, 1,5-pentanediol and 1,2-hexanediol). For each solvent, 20% (w/w) of water was added. The phenolic content of the extracts was quantified by HPLC-DAD, the antioxidant activity by the ORAC assay and the cytotoxic properties were assessed for tumor (HeLa) and non-tumor (PLP2) cell lines. The extracts obtained with 1,3-propanediol (PPD) achieved the best overall results. Afterwards, mixtures of PPD+betaine+water or PPD+choline chloride+water (binary and ternary mixtures) were studied as solvents, exploring the existing liquid phase region. In this context, an experimental design, assisted by response surface methodology, was developed to maximize the extraction yield of phenolic compounds. It was found that, under optimized conditions, similar extraction yields (Y) are obtained with the following composition of the mixtures: PPD+water (0.78:0.22, w/w) with Y = 26.0±0.4 mg/g dw; PPD+betaine+water (0.23:0.47:0.30, w/w) with Y = 26.4±0.1 mg/g dw; and, PPD+choline chloride+water (0.16:0.65:0.19, w/w) with Y = 25.1±0.2 mg/g dw. The chromatographic records (HPLC-DAD) presented a similar phytochemical profile, considering the quantification of the main phenolic compounds (3-O-caffeoylquinic acid, trans 3-p-coumaroylquinic acid, quercetin-3-O-glucoside and quercetin-O-pentoside) (Figure 1). Nevertheless, the results concerning the bioactivity of the extracts were significantly different. In fact, the eutectic extracts PPD+betaine+water (HeLa cell lines: GI50 = 103±3 µg/mL; PLP2 cell lines: GI50 = 158±6 µg/mL) and PPD+choline chloride+water (HeLa cell lines: GI50 = 80±1 µg/mL; PLP2 cell lines: GI50 = 131±1 µg/mL) have shown a higher cytotoxic potential compared to the binary mixture of PPD+water (HeLa cell lines: GI50 = 285±13 µg/mL; PLP2 cell lines: GI50 = > 500). The anti-inflammatory potential was also evaluated using a methodology based on the inhibition of the nitric oxide production by macrophages. Besides the absence of toxicity for the

non-tumor PLP2 cells, the PPD+water extract also did not present anti-inflammatory potential (EC50 > 500 µg/mL). In contrast, concentrations of EC50 =183±9 µg/mL and 134±1 µg/mL were obtained for betaine and choline chloride-based solvent extracts, respectively. Furthermore, no bioactivity of the pure solvents (binary and ternary mixtures) was observed under the experimental conditions (up to 4% of solvent). Therefore, small differences in the phytochemical profiles as well as a synergistic effect between extracts and solvents should explain the differences found.

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FIGURES

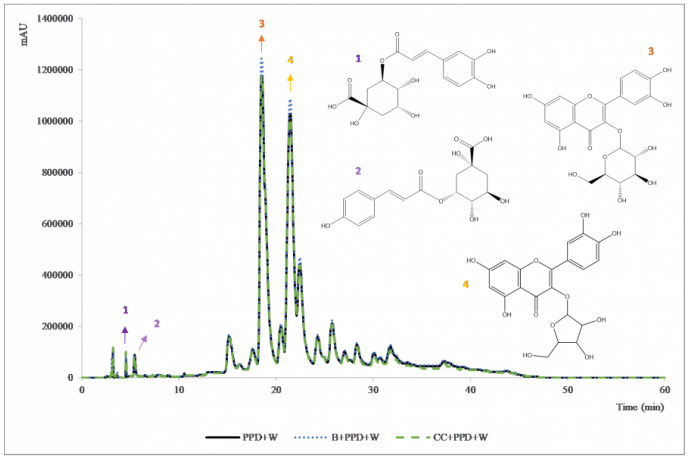

FIGURE 1

Chromatographic records at 370 nm for the quantification of the main phenolic compounds in J. regia L. leaves samples: 3-O-caffeoylquinic acid (1), trans 3-p-coumaroylquinic acid (2), quercetin-3-O-glucoside (3) and quercetin-O-pentoside (4).

FIGURE 2

KEYWORDS

Eutectic mixtures | walnut leaves | phenolic compounds | bioactivity

BIBLIOGRAPHY